Vol. 78, B, Part I (January to March) 2008 ISSN 0369-8211

Proceedings of the National Academy of Sciences, India

Section B - Biological Sciences

Published by
The National Academy of Sciences, India
5, Lajpatrai Road, Allahabad-211002

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, INDIA
(SECTION - B, BIOLOGICAL SCIENCES)
EDITORIAL BOARD

CONTENTS

Proc. Nat. Acad. Sci. India, Sect. B, Vol. 78 Pt. I, 2008

A comprehensive survey of the Pteridophytes of the Kangra district of Himachal Pradesh (India) has been carried out for the first time. Based on personal collections, reports in literature and study of herbarium specimens 130 species are being recorded. Three natural fern hybrids have been collected from this district. Three fern species are being reported for the first time from Himachal Pradesh.

Ethanolic turmeric extract (ETE) leads to inhibition and scavenging of strong oxidant, such as superoxide (O_2^-), hydrogen peroxide ($H_2O_2^-$) and hydroxyl radical (OH^-), both in case of lymphocytes as well as tumor cells. At the same time ethanolic turmeric extract was found to stimulate the lymphocytes to increase nitric oxide (NO) production.

Parkinsonia aculeata L. (Caesalpinaceae), a small xerophytic tree species, flowers twice a year, during March-April and August-September with profuse flowering in the former period. The flowers, arranged in lax axillary racemes are yellow, hermaphrodite, hypogynous, zygomorphic and complete. Temperature and relative humidity have direct effect on pollen viability, which is low during March-April and high during August-September.

	AMF association					
Crop interval of collection (DAS)	Spore Population 100gm^{-1} soil	% Colonization	Intensity of arbuscules cm^{-1} root length	Intensity of vesicles cm^{-1} root length	Total No. of AM spp.	Name of AM species
40	813	60	+++	-	15	ABRT, ASCB, CGRG, CHTG, GABD, GRSA, LAGR, LCLD, LCLR, LFSC, LHOI, LHTS, LINR, LMSS, LOCT
80	320	29	-	+	9	ABRT, ASPN, LAGR, LCLR, LFSC, LHOI, LHTS, LMSS, LOCT
120	996	75	-	+++	22	ABRT, ADLC, ASCB, ASPN, CGRG, CHTG, LABS, LAGR, LCLD, LCLR, LDMR, LDST LFSC, LHOI, LHTS, LINR, LMSS, LMST, LOCT, LPST, LRBF, LSNS

In the present study the occurrence of AM fungi on rice plants at varying stages of growth was observed. At the seedling stage (40 DAS) 15 AMF species, 60% root colonization and arbuscules in good amount were observed. But at maturation stage (80 DAS) when plants were submerged in water, not only species of AMF reduced but also per cent of root colonization decreased and no arbuscules were seen.

Pachytene karyotypes in fourteen accessions, belonging to four species of the genus *Carthamus* were analyzed in detail for establishing the chromosome and karyotype polymorphism. The karyotype and heterochromatin distribution patterns reported in this study provide a foundation toward cytological characterization of the *Carthamus* genome.

Arbuscular mycorrhizal (AM) fungi and their effect on growth of *Acacia catechu* seedlings was studied in relation to pesticide application. Three broad spectrum pesticides viz: a fungicide, a nematicide and an insecticide were applied in all possible combinations to remove maximum possible number of target organisms from the soil.

Natural populations of *Strychnos nux-vomica* Linn. in Eastern Ghats region produce heavy bloom during February - April. The flowers open in the evening hours during 1500h- 2000h, with a peak at 1600h - 1800 h. A syrphid fly *Sphaerophoria indiana* and three bee species *Xylocopa latipes* (juveniles), *Apis cerana indica* and *Trigona iridipennis* are the pollinators. *S. nux-vomica* seems to have adapted a mixed mating system with cryptic self-incompatibility.

No. of plantlets produced & transferred to pots	No. of surviving plants after 60 days	Survival percent (%)
30	28	93.3
39	38	97.4
32	29	90.0
41	39	95.12
40	38	95.0

'Average = 95%

Sweet Basil *(Ocimum basilicum)* is an important medicinal herb and is called 'Medicine Mughal' by many. It is used to prepare herbal formulations to cure different diseases. Its mosquito repellant property can also be exploited as an alternative to synthetic repellants used presently. An efficient protocol for *in vitro* shoot multiplication of Sweet Basil has been developed.

Table 4 - Component wise and total net primary production (t ha^{-1} $year^{-1}$) of *Acacia auriculiformis* plantation stand.

Year	Bole	Branch	Phyllode*	Root**	Total
Site-I					
1995-1996	14.31	3.94	2.93	2.28	23.54
1996-1997	16.32	4.82	3.43	2.43	27.00
Site-II					
1995-1996	14.99	7.09	1.31	1.51	24.90
1996-1997	12.55	5.56	2.07	1.04	21.22

Acacia auriculiformis A.Cunn. ex. Benth., a fast growing N_2-fixing tree, a native of Papua New Guinea, Australia, has come to India recently after its highly successful tree farming in Indonesia. At and around Varanasi, two age group plantations of *Acacia auriculiformis* stand as Site I & II. They have been studied for biomass production of bole, branch, phyllode and below ground parts.

$$\text{Abundance (\%)} = \frac{\text{Number of isolates of a particular fungal species}}{\text{Total number of fungi isolated}} \times 100$$

Survey of microfungi existing on the fruit surface of apple cv. Red Delicious was carried out during post-harvest phase. Relative importance of surface micromycetes in causing spoilage of apple fruits was also assessed. During the survey, a total of 32 microfungi belonging to 20 genera were isolated by surface washing technique. Artificial inoculations with the recovered surface mycopropagules were performed in apple fruits and most of them were able to cause fruit rot with different intensity.

PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES, INDIA
2008

VOL LXXVIII SECTION-B PART I

Diversity in the pteridophytes of Kangra district (Himachal Pradesh)

S.P. KHULLAR, SARIKA SHARMA and I.B. PRASHER

Department of Botany, Punjab University, Chandigarh-160014, India.

Received September 4, 2006, Revised February 20, 2007, Accepted March 16, 2007

Abstract

.... A comprehensive survey of the Pteridophytes of the Kangra district of Himachal Pradesh (India) has been carried out for the first time. Based on personal collections, reports in literature and study of herbarium specimens 130 species are being recorded. In Kangra district 45 species of Pteridophytes are rare; 22 are uncommon; 17 are occasional; 20 are very common: 14 are common and 12 are fairly common. Three natural fern hybrids have been collected from this district. Three fern species are being reported for the first time from Himachal Pradesh.

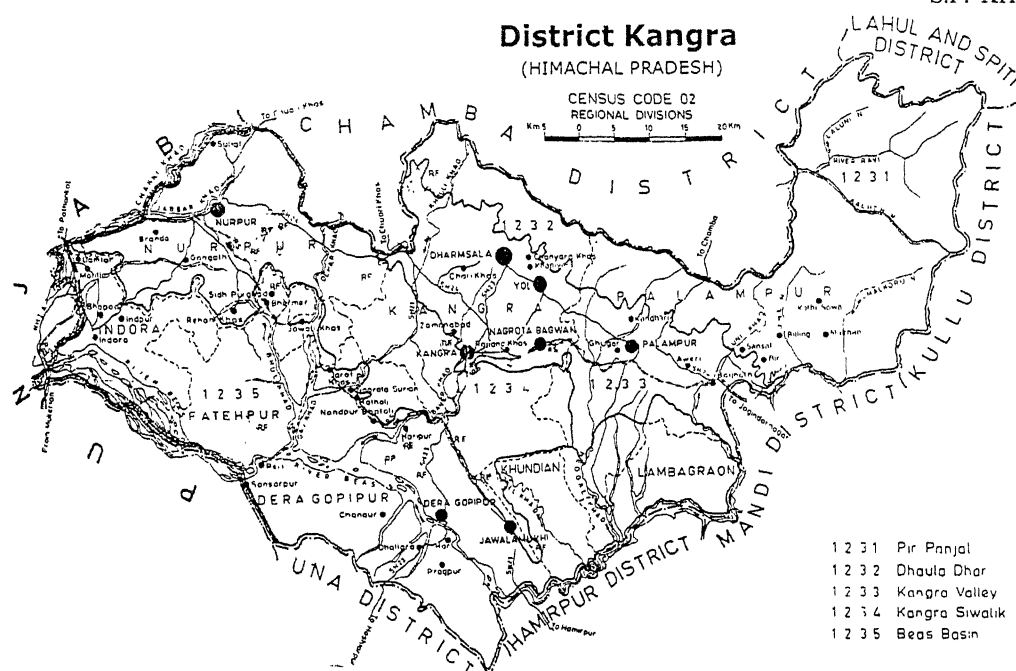
Key words: Pteridophytes, habitat, fern hybrids, biodiversity, Kangra district

सारांश

हिमाचल प्रदेश (भारत) के कांगड़ा नगर के टेरिडोफ़ाइट का प्रथम व्यापक सर्वेक्षण किया गया है। इस अध्ययन में जो, व्यक्तिगत संग्रह, प्रकाशित विवरण तथा वनस्पति संग्राहलयों पर आधारित है, 130 स्पीसीज़ लिपिबद्ध की गई हैं। कांगड़ा नगर में 45 स्पीसीज़ दुर्लभ, 22 असामान्य, 17 अवसरिक, 20 अत्यधिक सार्वजनिक, 14 सार्वजनिक तथा 12 अतिसार्वजनिक है। इस नगर से तीन प्राकृतिक फ़र्न संकर प्राप्त किये गये हैं। इस प्रदेश से तीन फ़र्न स्पीसीज़ का सर्वप्रथम विवरण दिया गया है।

सांकेतिक शब्द : टेरिडोफ़ाइट, निवास स्थान, फ़र्न–संकर, जैवविविधता, कॉगड़ा नगर।

Introduction

The Kangra district of Himachal Pradesh harbours an enormous diversity of flora. This region had strangely escaped attention of fern taxonomists and is almost unexplored for Pteridophytes. Victor Jacquemont, a Frenchman who came to India in 1829, got permission and access to explore the vegetation of Kangra and Kashmir from the then ruler of Punjab, Maharaja Ranjit Singh. But Jacquemont spent most of his time in Kashmir concentrating on plant geography Desmond R.[1]. E.W. Trotter made collections between (1886-1887), while Aitchinson (1879) collected a single fern (*Adiantum capillus-veneris* L. under *A.wattii* Baker) and Edgeworth (without date and locality) had a *Lepisorus*. The collections of all these collectors and a few others find mention in E.W. Trotter and C.W. Hope's (1890) *"Catalogue of Ferns in the Government Herbarium, Saharanpur"*. There are in all 14 such entries, of which seven are from Dharamsala. Some reports of Pteridophytes of Kangra district can also be found in the works of (Beddome, Clarke, Hope, Schelpe)[2-5]. Earlier Kangra was a large district and included district of Kulu as one of its tehsils. Therefore, most of these reports pertain to the present Kulu district. An account of the ferns of Dharamsala hills was published about three decades ago (Dhir and Datta)[6-9]. Some reports of ferns of Kangra district have also been included in Khullar[10] and Chandra[11]. But no comprehensive account of the Pteridophytes of the present day Kangra district exists. To remove this shortcoming, a comprehensive account of Pteridophytes of Kangra district has been prepared based on personal collections, reports in literature and study of herbarium specimens. The

Map of the Kangra district (united Map Co., Delhi)

diversity amongst Pteridophytes, as it exists, is also discussed.

Kangra district (See Map) forms the north-western part of Central Himachal Pradesh. It lies between 31^0 40' - 32^0-25' north latitude and 70^0 35' - 77^0 5' east longitude of the Western Himalaya. The topography of the district is well defined by a series of almost parallel hill ranges that rise in height towards north-east and enclose valleys. The altitude varies from 420m to 6400m above mean sea level. The district has considerable diversity in its soil, physiography, topography and climatic conditions.

Exhaustive explorations of several localities, visited many times for field collections (varying from low to high altitudes) are: Kangra, Gaggal, Mataur, Pathiyar, Bhawarna, Gupt-Ganga, Khaniyara, Bir, Billing, Sansal, Deol, Lanode, Kandbari, Kothi-Kohr, Lohardi, Multhan, Gopalpur, Baijnath, Palampur and Satovari (See Map). It was found that the areas covered under the tehsils Dehra, Nurpur, and some parts of tehsil Kangra have few Pteridophytes compared to areas falling under Palampur tehsil that comprises the eastern part of Kangra district. While giving the distribution of species, for the most common species that are found throughout the district only the names of tehsils have been given.

In the appended list the 130 species of Pteridophytes being reported, are arranged according to the classification proposed by Khullar[12]. The genera within a family and species within a genus are arranged in alphabetical order. From the Kangra district, three hybrids *(Athyrium x septentrionaliocci-dentalibharatense, Dryopteris x wechteriana* and *Polystichum x jamunae)*[12] have been collected, and three species *(Athyrium fangii, A. kumaonicum,* and *Thelypteris bukoensis)* are the first time collections from Himachal Pradesh. Collection numbers (SS = Sarika Sharma) have been given. Earlier reports if any have also been cited for each species after a semi-colon. All collections have been deposited in the Herbarium, Panjab University, Chandigarh.

List of Pteridophytes from Kangra District

EQUISETACEAE

***EQUISETUM* L.** Sp. pl. 2: 1061 (1753).

***E. debile* Roxb. *ex* Vauch.,** Mem. Soc. Phys. Hist. nat. Geneve 1 (2): 387 (1822).

E. ramosissimum Desf. subsp. *debile* (Roxb. *ex* Vauch) Hauke, Amer. Fern J. 52: 33 (1962).

Habitat: Common along rivers, streams or wet places around 600m and above.

Kangra (SS-18), Bir (SS -129), Khaniyara (SS-314).

It is a low altitude species, up to 1.2m tall, stems irregularly branched, ribs prominent but not scarious

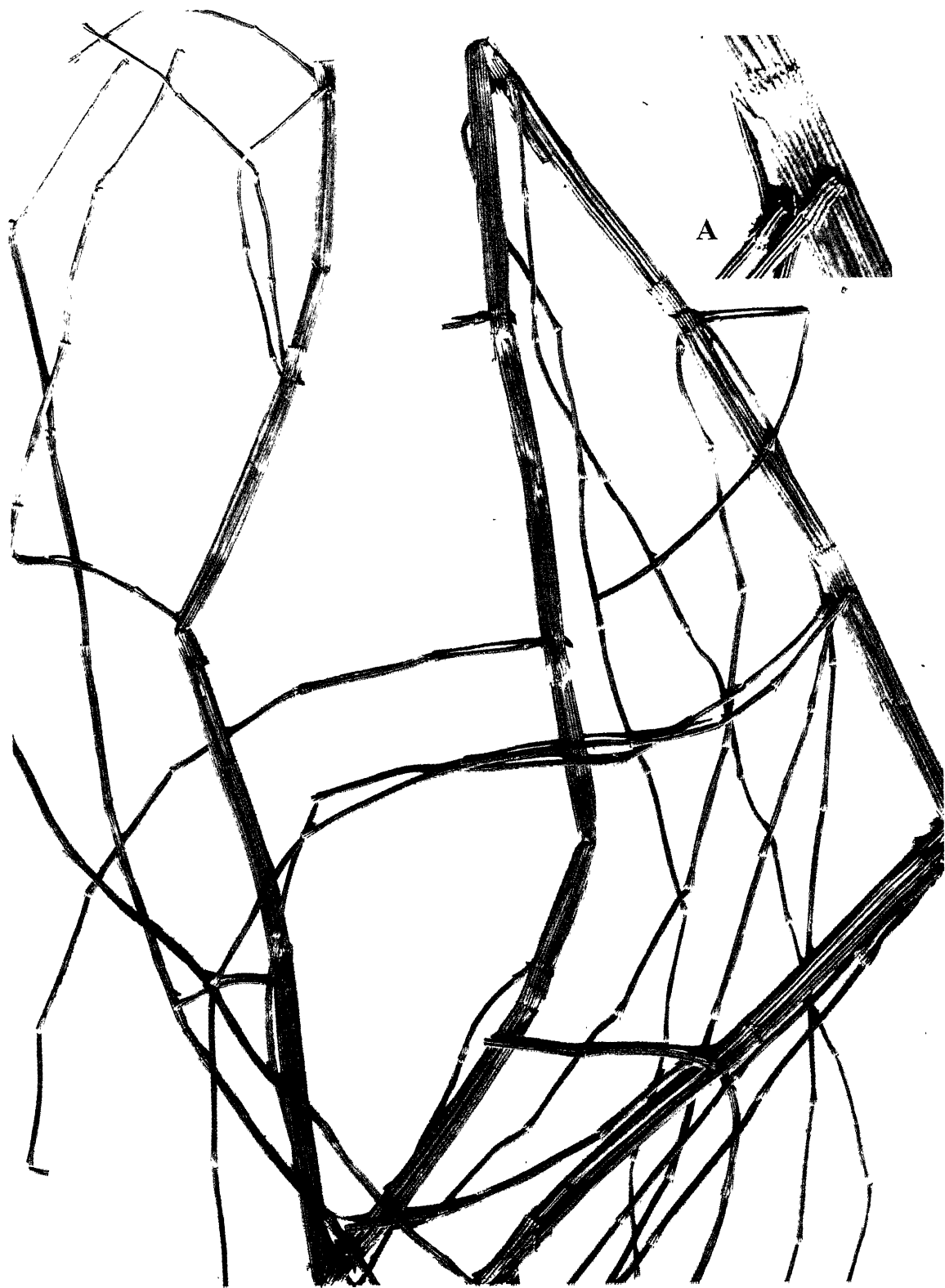



Plate 1
Equisetum ramosissimum
A. A part of internode magnified (x5).

Plate 2
Osmunda claytoniana
A. Fertile portion magnified (x3).

(as in *E. ramosissimum*); central cavity narrow; leaf sheath tight; leaf teeth lanceolate. (Plate 1).

***E. ramosissimum* Desf.,** Fl. Atlan. 2: 398 (1799).

Habitat: Occasional along ravine banks and water channels between 1200-1500m.

Khaniyara (SS-333).

Plants erect, up to 1.5m tall, 6-8mm in diameter; irregularly whorled; central cavity broad; ribs less prominent; leaf sheath loose; leaf teeth triangular.

These two species of *Equisetum* are often merged into a single species, *E. ramosissimum.*

SELAGINELLACEAE

***SELAGINELLA* Palisot de Beauv.,** Prodr. Fam. Aetheog. : 101 (1805) *nom. cons.*

***S. chrysocaulos* (Hook. & Grev.) Spreng.,** Bull. Acad. Roy. Sci. Belg. **10:** 232 (1843).

Lycopodium chrysocaulos Hook & Grev., in Hook. Bot. Misc. 2: 401 (1831).

Habitat: Very common along forest edges or along road-sides between 1000 - 2700m.

Sansal (SS-757), Bir (SS-160), Billing (SS-243), Multhan (SS-656).

Stems rooting at base; lateral leaves distant, denticulate.

OPHIOGLOSSACEAE

***BOTRYPUS* Michx.,** Fl. Bor.-Amer. 2: 274(1803).

***B. lanuginosus* (Wall. *ex* Hook. & Grev.) Holub,** Preslia **45:** 373 (1973).

Botrychium lanuginosum Wall. *ex* Hook. & Grev., Ic. Fil. 1: t.79 (1729).

Habitat: Rare around 2000m.

Lohardi (SS-974).

Common stipe long; sterile lamina large; fertile segment borne on a short stipe, much smaller than the sterile one, borne on a short stipe that usually arises above the base of the sterile lamina.

***OPHIOGLOSSUM* L.,** Sp. pl. 2: 1062 (1753).

***O. polyphyllum* A. Br, *ex* Seubert,** Fl. Azor.: 17 (1844).

Habitat: Occasional amongst grass in exposed and rather dry conditions up to 2400m.

Dharamsala: Chetru[6]

Sterile lamina usually linear lanceolate or ovate lanceolate; base of rhizome surrounded by brown persistent sheaths around the frond base.

OSMUNDACEAE

***OSMUNDA* L.,** Sp. pl. **2:** 1063 (1753).

***O. claytoniana* L.,** Sp. pl. 2: 1066 (1753).

Habitat: Uncommon at high altitudes above 2500m in meadows or on the forest-floor.

Kothi-Kohr (SS-435), Multhan (SS-618).

Lamina pinnate; middle 5-8 pairs of pinnae fertile, the rest above and below sterile. (Plate 2).

***O. regalis* L.,** Sp. pl. 2: 1065 (1753).

Habitat: Uncommon; inhabits wet surfaces in the forest around 2500m and above.

Kothi-Kohr (SS-475).

Fronds isomorphic; fertile pinnae terminal.

SCHIZEACEAE

***LYGODIUM* Sw.,** in Schrad. J. Botanik 1800(2):7,106 (1801) *nom. cons.*

***L. flexuosum* (L.) Sw.,** in Schrad. J. Botanik **1800** (2):106 (1801); **1800** (2): 304 (1802).

Ophioglossum flexuosum L., Sp. pl. 2: 1063 (1753).

Habitat: Rare amongst bushes; found up to 1500m. altitude.

Sidhwari[7].

Plants robust, climbers; pinnules large; sorophores marginal, protruding from the lamina margin.

DENNSTAEDTIACEAE

***DENNSTAEDTIA* Bernh.,** in Schrad. J. Botanik 1800(2):124 t.l (3) (1801).

***D. scabra* (Wall. *ex* Hook.) Moore,** Index Fil.: 307 (1861).

Dicksonia scabra Wall. *ex* Hook., Sp. Fil. 1: 80 t. 28 (1844).

Habitat: Rare on small cliffs or on steep slopes near waterfalls or deep sheltered stream gulleys around 2500m and above.

Multhan (SS-663), Komi - Kohr (SS-534).

Bir (SS-98), Billing (SS-227), Sansal (SS-786), Kandbari (SS-807), Multhan (SS-638); Dharamsala: Chetru, Dari[6].

Rhizome, long creeping; stipes thick, hairy when young later becoming rough due to fallen hair bases, stramineous but blackish at base; grooved on upper side; sori indusiate, usually in a sinus; indusia formed by the fusion of the true indusium and a minute tooth resulting in the formation of a cup-shaped or a slightly bivalved structure.

SINOPTERIDACEAE

***CHEILANTHES* Sw.**, Syn. Fil. **5:** 126 (1806) *nom. cons.*

C. anceps Blanf., Simla Nat. Hist. Soc. (25 June 1886).

C. farinosa Kaulf. var. *anceps* Blanf., Asiatic Soc. Bengal 57(4):306 (1888).

Habitat: Fairly common along forest slopes or rock surfaces around 1800m and above.

Bir (SS-191), Billing (SS-275), Sansal (SS-780), Khaniyara (SS-324); Dharamsala: Dari, Chetru, Chari.

Bicolorous scales running on entire stipe and rachis; stipes long, dark-ebenous to almost black; farina bright-white.

***C. bicolor* (Roxb.) Fras.-Jenk.,** Pak. Syst. 5(1-2): 94 (1991) [1992].

Pteris bicolor Roxb., in Griff. Calc. J. Nat. Hist. 4: 507 (1844).

Cheilanthes farinosa sensu auct. India, non (Forssk.) Kaulf.

Habitat: Very common on slopes, along road-sides on walls or in rock-crevices up to 1500m.

Kangra (SS-5), Nurpur (SS-895), Dehra (SS-925), Palampur (SS-49); Dharamsala: Chamunda Devi Temple[7].

Bicolorous scales generally restricted to stipe base or at most, present on entire stipe; rachis glabrous.

***C. brevifrons* (Khullar) Khullar,** Indian Fern J. **1**: 90 (1984).

C. anceps Blanf. var. *brevifrons* Khullar, Amer. Fern J. 66: 24 (1976).

Habitat: Uncommon along forest-slopes or on open slopes and road-sides between 1200-1800m. Bir(SS-190).

Fronds small; stipes thin, fragile; rachis scaly; lamina lower surface with a bright-white farina; upper surface of lamina dark-green; indusial margin highly lacerate with long projections.

***C. dalhousieae* Fras.-Jenk.,** Pak. Syst. 5: 89 (1991), *non* Hook. (1852), *et auct. plur.*

C. albomarginata Clarke (1880) *et auct. plur.*

The proposal of Xian-Chun Zhang (Taxon **51:** 381-382, 2002) to conserve the name of C. *albomarginata* for this species was rejected (Taxon **54** (3): 831-832, 2005).

Habitat: Common; inhabits humus rich habitats on forest-floor, rock-crevices or along forest-slopes between 1500 and 2500m.

Bir (SS-78), Billing (SS-281), Sansal (SS-782), Kandbari (SS-867), Multhan (SS-609), Khaniyara (SS-312); Dharamsala: Forsytheganj, Mcleodganj, Khanjjar Mahadev Temple [7] Stipe, primary secondary and tertiary rachis with bicolorous scales; farina pale or yellowish-white.

***C. leptolepis* Fras.-Jenk.,** Bot. Helv. **102:** 144 (1992).

C. *dalhousiae* Hook., Sp. Fil. 2: 80 t. 78 B (1852).

C.dalhousieae = C.dalhousiae named after its collector Lady Dalhousiae, is a correctable error under the code, Taxon 54: 831 (2005).

The committee rejected the proposal of Xian-Chun Zhang to conserve the name *C.dalhousieae* for this species (Taxon **54** (3): 831-832 (2005).

Habitat: Uncommon in rock-crevices in forest around 2500 m and above.

Sansal (SS-730); Dharamsala: Dharamkot[7].

Stipes brittle, covered with a few brown deciduous scales; farina completely absent at any stage of the development of the plant.

C. rufa **D. Don,** Prodr. Fl. Nepal :16 (1825).

Habitat: Rare; inhabits crevices of moist-lime rocks around 1400m.

Khaniyara(SS-341).

Stipe and rachis densely hairy (wooly) and sparsely scaly.

PELLAEA **Link,** Fil. Sp.: 59 (1841), *nom. cons.*

P. nitidula **(Wall. *ex Hook.*) Hook. & Bak.,** Syn. Fil.: 149 (1867).

Cheilanthes nitidula Wall. ex Hook., Sp. Fil. 2: 112 (1852).

Pteris nitidula Wall., Numer. List No. 89 (1828) *nom. nud.*

Habitat: Rare; inhabits rock-crevices between 1500 and 2400m.

Kangra[5].

Stipes dark-brown or blackish; indusia usually continuous along the margin of the lamina.

CRYPTOGRAMMACEAE

CRYPTOGRAMMA **R. Br. *ex* Richards.,** Bot. App. in Franklin's Journey Polar Sea : 767 (1823).

Cryptogramma brunoniana **Wall. *ex* Hook. & Grev.,** Ic. Fil. 2: t.158 (1829).

C. crispa sensu Beddome, Handb. Ferns Brit. India: 114(1883).

Habitat: Rare; inhabits rock-crevices around 2700m and above.

Dharamsala[10].

Fronds dimorphic; sterile pinnules obovate, base cuneate, margin deeply lobed, lobes toothed; fertile pinnules pod-like, linear, terminal pinnule as long as lateral ones.

ONYCHIUM **Kaulf.,** Jahr. d. Pharmacie Berlin 21 : 45 (1820).

O. contiguum Wall. ex Hope, J. Bomb. nat. Hist. Soc. **13:** 444 (1901).

O. cryptogrammoides Christ, Notul. Syst. [Paris] 1: 52-53 (1909).

Habitat: Fairly common on the humus rich forest-floor between 1500-3000m.

Bir (SS-62), Billing (SS-230), Sansal (SS-709), Kandbari (SS-805), Kothi-Kohr (SS-444), Khaniyara (SS-318), Satovari (SS-337); Dharamsala: Dharamkot[7]
Stipes stramineous but always black at base, fronds large; lamina finely dissected, pentagonal or spreading; sori grey at maturity.

Some consider *O. contiguum* to be a superfluous name for *O. japonicum* (Thunb.) O.Ktze. because, Hope not only cited *O. japonicum* var *multisectum* Henderson *ex* Clarke, in synonymy, but also and erroneously, *Leptostegia lucida* Ham. *ex* D. Don. The latter name was available for adoption at the specific rank under the rules even though it actually belongs to *O. japonicum,* a different species from what Hope intended to refer to. *O. contiguum* has therefore been renamed as *O. cryptogrammoides.,* Fraser-Jenkins [14]. In our opinion even though it may be against the rules, to avoid confusion that arises because of the indiscriminate name changes (to make them techinacally correct presumably!), it is better and makes common sense, to continue using the widely known names and make a strong case for their conservation. The mistakes in Hope may be corrected, and the name *O. contiguum* retained for this species.

O. lucidum **(D. Don) Spreng.,** Syst. Veg. 4: 66 (1827).

Leptostegia lucida D. Don, Prodr. Fl. Nepal : 14 (1825).

Habitat: Occasional on the humus rich soil of the forest-floor or in open along road-sides around 2000m and above altitude.

Bir (SS-171), Sansal (SS-776), Multhan (SS-632).

Stipes brown, glabrous, glossy; lamina sub-coriaceous, glossy; sori brown at maturity.

Fraser-Jenkins uses the name *O. japonicum* for this species.

HYPOLEPIDACEAE

HYPOLEPIS **Bernh.,** Schrad. neu. J. Bot. **1(2):** 34 (1806).

H. punctata **(Thunb.) Mett,** Kuhn Fil. Afr. : 120 (1868), *non sensu* Beddome (1892).

Polypodium punctatum Thunb., Fl. Jap. : 337 (1784).

Habitat: Very common; inhabits moist places along road-sides in open, forming large colonies between 1000 and 2500m

Bir (SS-108), Sansal (SS-711), Kandbari (SS-870), Multhan (SS-657), Palampur (SS-44), Jia (SS-31), Bandla (SS-34); Dharamsala: Khanjjar Mahadev Temple[7].

Rhizome long creeping; fronds large, up to 1-2m long, and 60 cm broad, hairy; sori partially or completely covered by a membranaceous suborbicular small reflexed margin of pinna lobe.

PTERIDIACEAE

PTERIDIUM **Gledich.** ***ex*** **Scopo.,** Fl. Carniol. : 169 (1760), *nom. cons.*

P. aquilinum **(L.) Kuhn var.** ***wightianum*** **(Ag.) Tryon,** Rhodora 43:22 (1941).

Pteris aquilina L., Sp. pl. 2: 1075 (1753).

Habitat: Occasional; forms thickets in open or in humus rich forests between 1800-2700m.

Kothi-Kohr (SS-529); Dharamsala [7]

Fronds large, subcoriaceous, densely hairy; sori marginal, continuous; indusia double.

PTERIDACEAE

PTERIS **L.,** Sp. pl. 2 :1073 (1753).

P. cretica L., Mant. Pl. : 130 (1767).

Habitat: Very common on the humus rich forest-floor or even in open or shaded localities between 1000 and 2500m.

Bir (SS-91), Sansal (SS-773), Kandbari (SS-802), Multhan (SS-604), Kothi-Kohr (SS-401); Dharamsala: Khanjjar Mahadev Temple, Dharamkot[7].

Fronds dimorphic; sterile fronds usually smaller, drooping; fertile fronds erect with longer and stronger stipes; lamina pinnate; pinnae long, margin spinulose serrate; lowest pair of pinnae usually forked at base.

P. excelsa **Gaudich.,** Freyc. Voy. Bot. : 388 (1827).

Habitat: Uncommon in shaded-damp places in the forest, near streams *etc.* around 2400m.

Multhan (SS-647), Lohardi (SS-987).

Fronds rather large; lamina 1-2 pinnate; pinnae at base well developed; basal pair of acroscopic and basiscopic veins anastomising to form a row of costal areolae.

P. pseudoquadriaurita **Khullar,** Illus. Fern Fl. West Himalaya 1: 272 (1994).

Habitat: A very common fern of the forest, also on forest edges between 1300 and 2000m.

Bir (SS-173), Billing (SS-246), Sansal (SS-767), Multhan (SS-629), Kothi-Kohr (SS-520), Khaniyara (SS-328); Dharamsala: Khanjjar Mahadev Temple[7].

Lowest pair of pinnae always the largest and forked at least once, rarely the second or third pairs of pinnae may also be similarly forked; the lowest pair of veins from either side of the costa reach the sinus but never fuse.

P. vittata **L.,** Sp. **pl.** 2: 1074 (1753).

Habitat: Very common along the streamlets, or river banks, on open roadsides, or on walls up to 2000m.

Kangra (SS-10), Nurpur (SS-892), Dehra (SS-942), Palampur (SS-42); Dharamsala[7].

Lamina pinnate; pinnae linear, with a truncate or cordate base or slightly auricled; sori marginal.

P. wallichiana **J. Ag.,** Recens. Sp. Gen. Pterid. : 69 (1839).

Habitat: Occasional in the forest or beds of dry streamlets or in moist situations or along roadsides between 1700 and 2700m.

Sansal (SS-768), Multhan (SS-635).

Stipes very long, castaneous, glabrous except at base, thick; lamina subpedate, generally 3 or 4-6 partite, two lateral and a central branch, each branch pinnate, large; two basal pairs of veins anastomosing to form a pair of areoles at base of costule, remaining veins free, forked.

ADIANTACEAE

ADIANTUM **L.,** Sp. pl. 2:1094 (1753).

A. capiltus-veneris **L.,** Sp. pl. 2: 1096 (1753).

Habitat: Very common up to 3000m in wet places, on walls, rocks, banks of stream, water courses, canals, walls of wells, wet slopes *etc.*

Kangra, (SS-2), Dehra, (SS-905), Nurpur, (SS-885), Palampur (S-47); Dharamsala: Chetru [7].

Lamina 2-3 pinnate; pinnae deltate, lowest pair of pinna the largest; pinnules or ultimate lobes sub rectangular and dimidate, outer margin of sterile lobes often irregularly lobed, 1/2 or 1/3 to the base into 3-5 obtuse shallowly lobed segments with a finely serrate-toothed margin. Plants without a proliferous terminal bud at the apex of an extended rachis.

A. edgeworthii **Hook.**, Sp. **Fil.** 2: 14, t. 81 B. (1851).

Habitat: Occasional in shaded humus rich forest slopes around 1600m and above.

Bir (SS-64); Dharamsala: Dari[7]

The lamina in the next three species is pinnate with the rachis usually extended with a proliferous bud at its tip and bearing small sized pinnae.

Lamina glabrous; pinnae small, many, triangular, dimidiate, shortly petiolate or nearly sessile, outer margin nearly straight or lobed into 3-5 truncate primary lobes with acute or subobtuse apex; sori on each lobe, never entire.

A. incisum **Forssk.**, Fl. Aeg. Ar. : 187 (1775).

Habitat: Very common along roadsides, in rock-crevices, on slopes and walls between 600 to 1200m.

Kangra (SS-7), Nurpur (SS-887), Palampur (SS-50), Dehra (SS-908); Dharamsala: Chari[7]

Lamina pinnate, hairy (as also are the stipe and rachis); pinnae dimidate or triangular, outer margin nearly straight or lobed into 3-5 primary truncate lobes.

A. philippense **L.**, Sp. pl. 2:1094 (1753).

A. lunulatum Burm., Fl. Indica : 235 (1768), *nom. superfl.*

Habitat: Fairly common besides streams, along roadside in humid and shaded areas between 800-1800m.

Kandbari (SS-810), Kangra (SS-6), Pathiyar (SS-11), Ranital (SS-906), Deol (SS-30); Dharamsala: Dharamsala K.B, Chetru [7]

Lamina pinnate, glabrous; pinnae lunate (semi-orbicular); outer margin of pinnae entire or shallowly lobed.

A. tibeticum **Ching & Y. X. Lin,** Acta Phytotax. Sinica **18** (1):104 (1960).

A. venustum sensu Fras.-Jenk., New Species Syndrome Indian Pteridology Ferns Nepal : 71 (1997) *non* D.Don.

Habitat: Uncommon; inhabits humus rich forest-floor or forest slopes around 2400m and above.

Bir (SS-139), Billing (SS-262), Sansal (SS-728), Kandbari (SS-863), Khaniyara (SS-370); Dharamsala: Mcleodganj, Khanjjar Mahadev Temple [7]

This is the octoploid of the *A. venustum* complex and is smaller in size with smaller pinnules but pinnules with long narrow acute teeth; grows at comparatively higher altitudes.

A. venustum **D. Don,** Prodr. Fl. Nepal : 17 (1825).

A. fimbriatum sensu Fras.-Jenk., New Species Syndrome Indian Pteridology Ferns Nepal : 309 (1997) *non* Christ.

Habitat: Common on forest- floor or along forest edges at mid-altitudes.

Bir (SS-106), Khaniyara (375).

This is the common mid-altitude species which is a tetraploid and with larger pinnules.

HEMIONITIDACEAE

CONIOGRAMME **Fee,** Gen. Fil. : 167 (1852) *nom. conser.*

C. ***affinis*** **Wall.** ***ex*** **Hieron.,** Hedwigia 57: 297 (1916).

Habitat: Rare; fern of the forest-floor around 2700m.

Kandbari (SS-848).

Lamina hairy; pinnae (or pinnule apex) gradually acuminate; hydathodes extended well into teeth.

C. caudata **(Wall.** ***ex*** **Ettingsh.) Ching,** in C. Chr. Index Fil. Suppl. 3: 56 (1934).

Grammitis caudata Wall. *ex* Ettingsh., Farnkr. 57: 38 (1864).

Habitat: Occasional along road-sides or on the forest-floor around 2400m in moist-shaded habitats.

Lohardi (SS-968).

Apex of pinnae or pinnules abruptly caudate, margin serrulate or sharply serrate.

C. indica **Fee,** Mem. Soc. sci. nat. Strasburg 10: 22 (1865).

Habitat: Common in moist-shaded localities on the forest-floor around 2400m.

Multhan (SS-694).

Lamina glabrous; pinna (or pinnule) apex slightly caudate; hydathodes reach tooth base only. (Plate 3).

C. intermedia **Hieron.,** Hedwigia 57: 310(1916) var. *intermedia.*

Habitat: Uncommon on the forest-floor above 2000m.

Lohardi (SS-964), Dharamsala[7].

Stipes stramineous; lamina texture thin, herbaceous, both surfaces pale-green; apex of pinna gradually and slightly caudate or gradually acuminate.

C. denticulatoserrata **(Hieron.) Dixit & Das,** Proc. Indian Acad. Sci. 88B pt II (4): 261 (1979).

C.fraxinea (D. Don) Fee *ex* Diels var. *denticulatoserrata* Hieron., Hedwigia 57: 290 (1916).

Habitat: Very rare; inhabits damp forest-floor/edges between 2000 and 2400m.

Multhan (SS-681).

Lamina pinnate or 2-pinnate at base, texture thick, chartaceous; pinnae base often unequal, rotundo-truncate, generally auriculate, margin distantly toothed with small teeth.

GYMNOPTERIS **Bernh.,** in Schrad. J. Botanik **1799(1):** 297 (1799).

G. vestita **(Wall.** *ex* **Moore) Underwood,** Bull. Torrey Bot. Cl. 29: 627 (1902).

Syngramma vestita Wall. *ex* Moore, Index Fil. : 60 (1857).

Habitat: Fairly common on exposed rocks around 1800m and above.

Billing (SS-240), Multhan (SS-610).

Lamina pinnate, both surfaces hairy, the lower densely covered with fine pale-brown or whitish hairs.

VITTARIACEAE

VITTARIA **J. E. Sm.,** Mem. Acad. Turin 5: 413 t. 9 (5), (1793).

V. flexuosa **Fee,** Mem. Fam. Foug. 3: 16 (1851-52).

Habitat: A very rare epiphyte between 1200 and 3000m.

Kangra valley[10].

Lamina simple, long, narrow, margin entire.

PARKERIACEAE

CERATOPTERIS **A. Brongn.,** Bull. Soc. Philom. Paris 1821: 186(1822).

C. thalictroides **(L.) A. Brongn.,** Bull. Soc. Philom. Paris **1821:** 186 (1822).

Acrostichum thalictroides L., Sp. pl. 2: 1070 (1753).

Habitat: Very rare in rice fields, ponds and ditches or marshy places up to 1000m.

Palampur, Dharamsala [9].

Fronds di-morphic; stipes spongy; sporangia in 2-3 longitudinal rows along the veins. An amphibious fern.

MARSILEACEAE

MARSILEA **L.,** Sp. pl. 2: 1099 (1753).

M. minuta **L.,** Mant. : 308 (1771).

Habitat: Very common in ditches, small ponds, rice fields, banks of lakes *etc.* from plains to mid-altitudes up to 1800m.

Gupt Ganga (SS-17); Dharamsala: Dari[7].

Aquatic fern; lamina quadrifid divided into four pinnae; sporangia enclosed in a pedicellate sorocarp with very thick walls.

THELYPTERIDACEAE

THELYPTERIS **Schmidel,** Icon. PL ed. Keller **3:** 45 t.[11], 13 (1763) *nom. cons.*

T. arida **(D. Don) Morton,** Amer. Fern J. **49:** 113 (1959).

Aspidiumn aridum D. Don, Prodr. Fl. Nepal : 4 (1825).

Cyclosorus aridus (D. Don) Ching, Bull. Fan Mem. Inst. Biol. (Bot.) **8** : 194 (1938).

Christella arida (D.Don) Holttum in Nayar & Kaur Comp. Bedd. Handb. Ferns Brit. India : 206 (1974).

Habitat: Rare along streamlets in shade between 1500 and 1800m.

Bhawarna (SS-29); Dharamsala: Dharamsala K.B.[8].

Lamina pinnate; pinnae margin very shallowly lobed distinctly half-way to the costa; 3-5 or more pairs of lower pinnae abruptly or subabruptly reduced.

***T. auriculata* (J. Sm.) K. Iwats.,** Acta Phytotax. Geobot. **19** : 11 (1961).

Phegopteris auriculata J. Sm., Hist. Fil.: 233 (1875).

Cyclogramma auriculata (J. Sm.) Ching, Acta Phytotax. Sinica 8: 317 (1963).

Habitat: Common along streamlets or in moist situations around 1200m and above up to 2700m.

Bir (SS-89), Sansal (SS-722), Khaniyara (SS-392), Palampur (SS-41); Dharamsala: Dharamsala K.B.[8].

Stipes, rachis and lamina with acicular hooked hairs; aerophores at base of pinnae on lower surface prominent and curved; lower pinnae gradually reduced, still lower ones abruptly or gradually reduced to auricles.

***T. bukoensis* (Tagawa) Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) **6** : 272 (1936).

Dryopteris bukoensis Tagawa, Acta Phytotax. Geobot. **1** : 89 (1932).

Habitat: Very rare along water-courses, streamlets along road-sides between 2000 and 2400m.

Multhan (SS-634).

Pinnae rather long, oblong, many segments lobed half-way to costule, many very small scales, each with a tuft of hairs, on lower surface.

***T. dentata* (Forssk.) E. St. John.,** Amer. Fern J. **26** : 272 (1936).

Polypodium dentatum Forssk., Fl. Aegypt. Arab.: 185 (1775).

Cyclosorus dentatus (Forssk.) Ching, Bull. Fan Mem. Inst. Biol. (Bot.) 8: 206 (1938).

Christella dentata (Forssk.) Brownsey & Jermy, Brit. Fern Gaz. **10** : 338 (1973).

Habitat: Very common along river banks, water courses, moist ditches *etc.* between 400-2000m.

Bir (SS-170), Sansal (SS-720), Kothi-Kohr (SS-441), Kangra (SS-8), Nurpur (SS-897), Dehra (SS-946), Palampur (SS-52), Andreta (SS-55), Pathiyar (SS-14), Gopalpur (SS-43); Dharamsala: Dharamsala K.B., Dari, Chari[8].

Lower 2-4 pairs of pinnae reduced and auricled only on the acroscopic side the auricle well developed and enlarged in the lower pinnae; pinnae margin lobed about 1/2 or a little more to the costa; lobes regular, slightly oblique, subfalcate, rounded.

***T. erubescens* (Wall. *ex* Hook.) Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) 6: 293 (1936).

Polypodium erubescens Wall. *ex* Hook., Sp. Fil. **6** : 236 (1862).

Glaphyropteridopsis erubescens (Wall. *ex* Hook.) Ching, Acta Phytotax. Sinica **8** : 320 (1963).

Habitat: Common along water-falls, near ravines, or along road-sides between 800m and 2700m.

Bir (SS-193), Sansal (SS-750), Kandbari (SS-860), Multhan (SS-687), Kothi-Kohr (SS-419); Dharmsala: Dharamkot[8].

A large-sized fern; lamina pinnate; swollen pale aerophores at base of pinnae, lower 1-3 pairs of pinnae strongly downwards deflexed, the lowermost pinnae narrowed at base; sori contiguous, close to the costule.

***T. papilio* (Hope) K. Iwats.,** Mem. Coll. Sci. Univ. Kyoto B **31**: 175 (1965).

Nephrodiumpapilio Hope, J. Bomb. nat. Hist. Soc. **12**: 625 (1899); **14**: 747 (1903).

Cyclosorus papilio (Hope) Ching, Bull. Fan Mem. Inst. Biol. (Bot.) **8**: 214 (1938).

Christella papilio (Hope) Hollt., in Nayar & Kaur Comp. Bedd. Handb. Ferns Brit. India : 208 (1974).

Habitat: Uncommon along streamlets and water-channels in shade up to 1600m.

Khaniyara (SS-315); Dharmsala: Dari[8].

Lamina texture thin, membranaceous or papyraceous; lower 5-10 pairs of pinnae gradually distant and much shortened and becoming 'triangular' in shape due to both acroscopic and basiscopic lower lobes being well developed and assuming a different shape from the pinnae in the middle of the frond.

***T. parasitica* (L.) Tard.-Blot.,** Notul. Syst. 7: 75 (1938).

Polypodium parasiticum L., Sp. pi. **2** : 1090 (1753).

Cyclosorus parasiticus (L.) Farwell, Amer. Midi. Nat. 12: 259 (1965).

Christella parasitica (L.) Leveille, Flore Kouy-Tcheou : 475 (1915).

Habitat: Rare along water courses or in moist places up to 1500m.

Bhawarna (SS-35).

Rhizome long creeping; stipes as long as the lamina, softly hairy; lamina lower surface hairy, hairs up to 1mm long; thick orange or yellow glands normally present on lower surface of veins.

***T. penangiana* (Hook.) C. Reed,** Phytologia **17**: 303 (1968).

Polypodium penangianum Hook., Sp. Fil. 6: 13 (1863).

Abacopteris penangiana (Hook.) Ching, Bull. Fan Mem. Inst. Biol. (Bot.) 8: 253 (1938).

Pronephrium penangianum (Hook.) P. Chandra. Bull. Bot. Surv. India **13:** 274 (1971).

Habitat: Rare near water flushes, streams, gulley and sometimes on cliffs near water falls up to 1800m.

Kangra [10].

Lamina pinnate; pinnae many pairs, long and broad (up to 3.5 cm broad); sori exindusiate.

***T. prolifera* (Retz.) C. Reed,** Phytologia **17** : 306 (1968).

Hemionitis prolifera Retz., Obs. Bot. **6** : 36 (1791).

Ampelopteris prolifera (Retz.) Copel., Gen. Fil.: 144 (1947).

Habitat: A very common low altitude thicket forming fern found in the plains along river banks, water courses *etc.* between 1600 and 2400 m.

Kangra (SS-4), Nurpur (SS-880), Dehra (SS-935), Pathiyar (SS-10), Gaggal (SS-21); Dharmsala: Chetru[8].

Rachis of indefinite length, hairy, hairs unicellular or forked or simple, long, often flagelliform; axillary proliferous buds present.

***T. pyrrhorhachis* (Kunze) Nayar & Kaur,** Comp. Bedd. Handb. Ferns Brit. India: 72 (1974) subsp. ***distans* Fras.-Jenk.,** New Species Syndrome Indian Pterid. Ferns Nepal : 21 (1997).

Phegopteris distans (D. Don) Mett., Abh. Mett. Senckenb. Naturf. Ges. 2. *Pheg. Aspl.* :16 (1858).

Habitat: Common along streams, watercourses in humus rich places between 1600 and 2400 m.

Multhan (SS-660), Kothi-Kohr (SS-581); Dharamsala: Dari[8].

Rhizome short-creeping or suberect; fronds arise close together; lamina 1 -2 (-3) pinnate, large, margin of ultimate segments crenately lobed or pinnatifid half-way to costule, but progressively less lobed to almost entire; a diploid sexual.

T. pyrrhorhachis* Nayar & Kaur,** Comp. Bedd. Handb. Ferns Brit. India: 72 (1974) subsp. ***pyrrhorhachis.

Polypodium pyrrhorhachis Kunze, Linnaea **24** : 257 (1851).

Pseudophegopteris pyrrhorhachis (Kunze) Ching, Acta Phytotax. Sinica **8** : 215 (1963).

Thelypteris brunnea Wall. *ex* Ching, Bull. Fan Mem. Inst. Biol. (Bot.) **6** : 296 (1963).

Habitat: Rare; inhabits wet places along streamlets in the forest or along road-sides between 2000 and 2700m.

Multhan (SS-623).

Rhizome long-creeping; fronds distantly placed. This is a tetraploid sexual.

***T. repens* (Hope) Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) **6** : 304 (1936).

Plate 3
Coinogramme indica
A. A part of pinna magnified (x 3).

Plate 4
Athyrium fangii
A. A pinna magnified (x 5).

Nephrodium repens Hope, J. Bomb. nat. Hist. Soc. **12:** 535 (1899).

Nephrodium cannum Bak., Syn. Fil. : 267 (1867).

Pseudocyclosorus cannus (Bak.) Hollt. & Grimes, Kew Bull. **34:** 509 (1979).

Habitat: Common in the forest near streams and often also on damp road-sides from 1000-1800m.

Bir (SS-127), Sansal (SS-734), Kandbari (SS-815), Baijnath (SS-32), Bhawarna (SS-24); Dharmsala: Dari, Chetru[8].

Rhizome long-creeping; stipes and rachis circular; aerophores moderately swollen, not prominent; reduced pinnae between 6-10 pairs, all with a distinct green lamina.

***T. totta* (Schlecht.) Nayar & Kaur,** Comp. Bedd. Handb. Ferns Brit. India : 90 (1974), *non* Thunbg.

Gymnogramma totta Schlecht., Adumbr.: 15 (1825).

Stegnogramma mollissima (Kunze) Fras.-Jenk., New Species Syndrome Indian Pterid. Ferns Nepal: 237 (1997).

S. pozoi (Lagasca) K. Iwats., Acta Phytotax. Geobot. **19** : 124 (1963).

Habitat: Rare along streams or occasionally amongst rocks in shade or by streams or in rock crevices between 1800 and 2400m.

Multhan (SS-631), Kothi-Kohr (SS-415).

Lamina pinnate; all axes hairy; sori exindusiate, elongate, running along veins, sometimes also on excurrent veins, sporangia bearing numerous slender setae.

***T. tylodes* (Kunze) Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) **6** : 286 (1936).

Aspidium tylodes (err. *xylodes)* Kunze, Linnaea **24:** 244, 283 (1851).

Habitat: Fairly common; inhabits moist habitats often near waterfalls or cliffs or open habitats between 1500 and 2000m.

Bir (SS-100), Sansal (SS-713), Khaniyara (SS-306); Dharamsala: Bhagshu Nath Fall[8].

Rhizome erect; rachis quadrangular; lamina pinnate; large, many pairs of lower pinnae gradually reduced, lowest represented by dark coloured aerophores on stipe without green lamina, sometimes abortive, intermediate type of pinnae represented by tubercle-like vestiges.

ATHYRIACEAE

ATHYRIUM Roth, Tent. Fl. Germ. **3** : 31, 58 (1799).

***A. anisopterum* Christ,** Bull. Herb. Boissier **6** : 692 (1898).

Habitat: Rare on moss covered wet rock surfaces or on moist dripping rocks in shade between 1500 and 2000m.

Kothi-Kohr(SS-491).

Plants small to medium-sized; pinnae with rectangular bases and obtuse apices, shallowly lobed to pinnate; lobes widely obtuse with narrow obtusely toothed lobes; diploid sexual.

***A. atkinsonii* Bedd.,** Suppl. Ferns Southern India and Brit. India : 11 t. 359 (1876).

Habitat: Rare; inhabits moist humus rich habitats between 2400 and 3000m.

Dharamsala: Triund[6].

Rhizome thin; fronds arise at distant intervals; rachis glabrous or sparsely scaly; pinnules and ultimate segments symmetrical about their axes.

***A. attenuatum* (Wall. *ex* Clarke) Tagawa,** Acta. Phytotax. Geobot. **16** : 177 (1956).

Asplenium filix-femina Bernh. var. *attenuata* Clarke, Trans. Linn. Soc. London 2 Bot. **1** : 492 (1880).

Habitat: Rare; inhabits exposed rocky forest margins above 3000m altitude.

Dharamsala: Triund[6].

Stipe and rachis pale; pinnae deeply lobed sometimes becoming pinnately lobed, not auriculate at their bases, lowest few pairs of pinnae not much reduced or not reduced at all.

***A. distans* (D. Don) Moore,** Index Fil. : 125 (1859).

Asplenium distans D. Don, Prodr. Fl. Nepal: 9 (1825) *non* Fee *nec* Brack.

Habitat: Uncommon in the forests, along banks of streams between 1500-2500m.

Kothi-Kohr(SS-575).

Lamina widest just above the base, thinly herbaceous; no proliferous buds; pinnules triangular lanceolate, deeply lobed into well separated lobes with the apices of these lobes bearing short insignificant acute teeth.

***A. fangii* Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) (*n.s.*) **1(3)** : 282 (1949).

Habitat: Rare along banks of streams or water courses up to 2500m.

Multhan(SS-672).

Similar to *A. distans* but differing from that species in having a thick lamina in contrast to the thin herbaceous one in *A. distans.* (Plate 4).

***A. fimbriatum* Moore,** Index Fil. : 185 (June 1860), *non* Dulac (1867).

Habitat: Uncommon in damp forest or along streamlets or along waterfalls in shade between 2000 and 3500m.

Kothi-Kohr (SS-474); Dharamsala: Triund[6].

Rhizome long-creeping; stipes arise close together; lamina large to huge; pinnae sloping, obliquely inserted, markedly asymmetrical, acroscopic pinnules larger than the basiscopic ones.

***A. flabellulatum* (Clarke) Tard. Blot.,** *Asplen.* Tonkin : 85 t. 13 (1932).

Asplenium filix-femina Bernh. var. *flabellulatum* Clarke, Trans. Linn. Soc. London 2 Bot. **1** : 493 (1880).

Habitat: Very rare in the moist-shaded conditions of the forest around 2700m and above.

Kothi-Kohr (SS-582).

Stipes scaly at base, rest glabrous; lamina 2-pinnate, lanceolate; margin of pinnae lobed less than half to the costa into deeply incised lobes.

***A. foliolosum* Wall, *apud* Moore *ex* Sim,** Priced Cat. Ferns 6: 22 (1859).

Habitat: Uncommon on slopes near waterfalls or crevices of moist rocks around 2400m. Multhan (SS-649), Kothi-Kohr (SS-523).

Stipe and rachis yellowish in the living state; lamina 1-2 pinnate, deltate-lanceolate, membranaceous when dried; margin deeply lobed to the costa (or becoming pinnate); sori large with prominent indusia.

***A. kumaonicum* Punetha,** Indian Fern J. **2** : 29 (1985).

Habitat: Very rare in wet habitats along water courses around 1800m.

Multhan (SS-620).

Plants rather large; stipes stramineous or pink, glabrous but scaly at base; lamina 2-pinnate, thin herbaceous; setae absent on adaxial surface.

***A. mackinnoniorum* (Hope) C. Chr.,** Index Fil. **1** : 143 (1905).

Asplenium mackinnoniorum [mackinnoni] Hope, J. Bot. London **34:** 124 (1896).

Habitat: Uncommon in moist conditions in the forest around 2000m and above.

Sansal (SS-773); Dharamsala: Dari[6].

A fern of higher altitudes with large wide spreading deltate fronds; rachis costa and costules glabrous except for setae confined to pinna apices.

***A. micropterum* Fras.-Jenk.,** New Species Syndrome Indian Pterid. Ferns Nepal : 58 (1997).

Habitat: Rare on wet moss-covered rocks or damp shaded cliffs, or even in open river gorges or in meadows around 2200m and above.

Multhan (SS-607), Kothi- Kohr (SS-536).

Plants small with thin stramineous or green stipes; lamina pinnate, small; pinnae margin shallowly lobed; sori indusiate; indusia large.

***A. pectinatum* (Wall. *ex* Mett.) Moore,** Index Fil.: 152 (Nov. 1859), 186 (June, 1860), *non* Fee (1866).

Asplenium pectinatum Wall. *ex* Mett., Abhandl. Senckenb. Naturf. Ges. (Frankfurt) **3** : 241 (1859).

Habitat: Very common in the open or along the forest edges between 1000-1800m and above.

Bir (SS-174), Billing (SS-235), Sansal (SS-762), Kandbari (SS-833), Khaniyara (SS-336); Dharamsala: Forsytheganj, Mcleodganj, Bhagshu Nath[6].

Plate 5
Athyrium strigillosum
A. A portion of lamina magnified (x 5).

Plate 6
Diplazium maximum
A.A. portion of a pinna magnified (x3).

Rhizome thin, creeping; lamina very finely dissected into long, narrow oblong ultimate lobes. Since the lobes are very narrow and small, the sori are also small.

A. schimperi **Moug.** *ex* **Fee.** Mem. Fam. Foug. 5 Gen. Fil. : 186-187 (1852).

Habitat: Fairly common on the forest-floor or along forest-edges in shade between 1200 and 2700m.

Bir (SS-117), Sansal (SS-736), Kandbari (SS-813), Kothi-Kohr (SS-457), Khaniyara (SS-329); Dharamsala: Forsytheganj, Mcleodganj, Khanjjar Mahadev Temple[6].

Stipes distant on rhizome, stramineous but always dark at base; lamina pinnate; lower 1-2 pairs of pinnae generally slightly reduced and distant.

A. x septentrionalioccidentalibharatense **Fras.-Jenk.,** in Khullar Illus. Fern Fl. West Himalaya, **2** : 83 (2000).

(A. pectinatum x A. schimperi)

Habitat: Rare in moist humus-rich shaded forest edge around 1800m and above.

Bir (SS-72).

This is a hybrid with morphology intermediate between its supposed parents.

A. setiferum **C. Chr.,** Index Fil. **1** : 146 (1905).

Habitat: Uncommon in shade along streamlets around 2700m and above.

Multhan (SS-602).

Stipes thin, fragile, with a blackish base; lamina 2-pinnate; pinnules small, elliptic or oblong rhombic, symmetrical, apex rounded with a few short teeth; costae and costules with scattered long weak setae on upper surface.

A. strigillosum **(T. Moore** *ex* **E.J. Lowe) Moore** *ex* **Salmon,** Nomenc. Gefasskrypt : 112 (1883).

Asplenium strigillosum T. Moore *ex* E. J. Lowe, Ferns Brit. Exot. **5** : 107-108 t 36 (1858). (Plate 5)

Habitat: Fairly common in wet places or along banks and beds of well-shaded ravines between 1300 and 2400m.

Bir (SS-184), Multhan (SS-655).

Stipe and rachis stramineous or pinkish, prominently grooved on upper side, ultimate lobes sharply serrate-dentate, the acroscopic basal pinnule generally the largest; proliferous buds in the axes of the upper pinnae. (Plate 4).

DEPARIA **Hook. & Grev.,** Ic. Fil. 2: 154 (1829).

D. allantodioides **(Bedd.) M. Kato,** Bot. Mag. Tokyo **90** : 35 (1977).

Athyrium allantodiodies Bedd., Ferns Brit. India: 221 (1867).

Lunathyrium allantodiodies (Bedd.) Ching, Acta Phytotax. Sinica **9** : 72 (1964).

Athyrium thelypteroides sensu Bedd., Handb. Ferns Brit. India : 164 (1883) *non* Michx.

Habitat: Rare; inhabits humus rich forest-floor along water channels between 1800 and 3600m.

Dharamsala: Triund[6].

Fronds large, with articulated hairs on stipe rachis costae and costules; lowest acroscopic pinnule on each pinna of the same size as or rarely slightly longer than the rest and hardly more lobed.

D. boryana **(Willd.) M. Kato,** Bot. Mag. Tokyo **90** : 36 (1977).

Aspidium boryanum Willd., Spec. pl. 5: 285 (1810).

Dryoathyrium boryanum (Willd.) Ching, Bull. Fan Mem. Inst. Biol. (Bot.) **11** : 81 (1941).

Habitat: Rare on the forest floor around 2400m.

Multhan (SS-645); Kangra (Clarke).

Fronds very large to huge; bipinnate to deeply tripinnatfid; sori round.

D. japonica **(Thunb.** *ex* **Murray) M. Kato,** Bot. Mag. Tokyo **90** : 37 (1977).

Asplenium japonicum Thunb. *ex* Murray, Syst. Veg.: 934 (1784).

Diplazium japonicum sensu Bedd., Ferns Brit. India Suppl.: 12 (1876) *pro parte.*

Lunathyrium japonicum (Thunb. *ex* Murray) Kurata, J. Geobot. **9** : 99 (1961).

Habitat: Very common along water channels or in wet, moist places between 1500 and 2400m.

Bir (SS-111), Sansal (SS-729), Multhan (SS-622), Kothi-Kohr (SS-547); Dharamsala: Chetru, Chamunda Devi Temple, Dharamkot[6].

Stipes often shorter than lamina which is triangular-lanceolate or subdeltate; ultimate lobes symmetrical, apex rounded with few short blunt teeth, margin subentire or finely serrate. A diploid sexual.

***D. petersenii* (Kunze) M. Kato,** Bot. Mag. Tokyo **90** : 37 (1977).

Asplenium petersenii Kunze, Anal. Pterid. : 24 (1837).

Diplazium petersenii (Kunze) Christ, Bull. Acad. Geogr. Bot. Mans. : 245 (1902).

Lunathyrium petersenii (Kunze) H. Ohaba, Sci. Rep. Yokosuka City Mus. **11** : 53 (1965).

Habitat: Common in shade along streamlets between 500 to 1200m.

Bir (SS-168), Bhawarna (SS-27), Mataur (SS-15).

Lamina triangular lanceolate, pinnate; ultimate lobes characteristically more rectangular with a broadish apex than in *D. japonica.* A tetraploid sexual.

***DIPLAZIUM* Sw.,** in Schrad. J. Botanik 1800(2): 4, 61 (1801).

***D. esculentum* (Retz.)** Sw., in Schrad. J. Botanik 1800 (2): 312 (1803).

Hemionitis esculenta Retz., Obs. Bot. **6** : 38 (1791).

Athyrium esculentum (Retz.) Copel., Philip. J. Sci. C.3 : 295 (1908).

Habitat: Very common on slopes, in fields, or even along roadsides between 400 to 1000m.

Kangra (SS-3), Dehra (SS-904), Nurpur (SS-882), Palampur (SS-48); Dharamsala[6].

Lamina size and pinnation variable depending upon the age of the plant; the basal 2-3 pairs of veinlets of adjacent lobes fuse to form an irregular excurrent veinlet which runs to the sinus forking just below it with one half going to each lobe almost to the margin, remaining 6-10 pairs arising from a central main veinlet in a segment free.

***D. maximum* (D. Don) C. Chr.,** Index Fil. **1** : 235 (1905).

Asplenium maximum D. Don, Prodr. Fl. Nepal : 8 (1825).

Diplazium giganteum (Bak.) Ching, in C. Chr. Index Fil. **3** : 73 (1934).

Diplazium frondosum (Clarke) Christ, Notul. Syst. [Paris] **1** : 46 (1909).

Diplazium polypodioides sensu auct. West Himalaya *non* Blume.

Habitat: Fairly common along streamlets or wherever water is in plenty, also in the forest between 1300 and 2500m.

Bir (SS-98), Billing (SS-227), Sansal (SS-786), Kandbari (SS-807), Multhan (SS-638); Dharamsala: Chetru, Dari[6].

Rachis glabrous; lamina 2-pinnate, huge; margin of ultimate segments minutely serrate; sori indusiate, stretching from the costa and almost reaching the margin. Often wrongly referred as *D. polypodioides* from Himachal Pradesh and many other localities in west Himalayan fern literature till recently. True *D. polypodioides* is found in Kumaun, E.Himalaya and S.India. (Plate 6).

***WOODSIA* R. Br.,** Prodr. Fl. Nov. Roll. : 158 (1810).

***W. elongata* Hook.,** Sp. Fil. ed. **1** : 62 (1844).

Habitat: Occasional on exposed rocks or on gravelly soil around 2500m and above.

Lohardi (SS-989); Dharamsala: Triund[6].

Lamina glabrous or hairy only along margin or costae and costules; pinnae many, 25-35 pairs, texture thick chartaceous; sori marginal, accessory indusium present.

HYPODEMATIACEAE

HYPODEMATIUM Kunze, Flora **16** : 690 (1833).

H. crenatum* (Forssk.) Kuhn *ex* v. Deck.,** Reis. Ostrafr. (Bot.) **3** : 37 (1879) subsp. ***crenatum.

Polypodium crenatum Forssk., Fl. Aeg. Arab. : 185 (1775).

Lastrea crenata (Forssk.) Bedd., Ferns Brit. India Suppl. : 18 (1876).

Habitat: Very rare in exposed conditions in rock crevices or on slopes around 1600m and above.

Bir (SS-172); Dharamsala: Chamunda Devi Temple[7].

Lamina 3-pinnate, deltate pentagonal, both surfaces hairy; stipes always with a cushion of golden brown hair at base.

DRYOPTERIDACEAE

***CYRTOMIUM* Presl**, Tent. Pterid.: 86 t. 2(26) (1836).

***C. caryotideum* (Wall. *ex* Hook. & Grev.) Presl**, Tent. Pterid. : 86, t 2(26) (1836).

Aspidium caryotideum Wall. *ex* Hook. & Grev., Ic. Fil. **1** : t 69 (1828).

Habitat: Rare in damp shaded localities along road-sides, or along waterfalls or streams between 1500 and 2100m.

Multhan (SS-696), Lohardi (SS 955).

Lamina pinnate; upper surface mid-green; pinnae large, strongly auriculate or bi-auriculate, acroscopic base extended into a long sharp auricle, the basiscopic side rounded or obliquely truncate at base; the lowest pinnae pair and the terminal pinna strongly auricled on both the sides, margin strongly serrate, teeth acute.

***DRYOPTERIS* Adanson,** Fam. pl. **2** : 20, 551 (1763) *nom. conser.*

***D. barbigera* (Moore *ex* Hook.) O. Ktze.,** Revis. Gen. pl. **2** : 812 (1891).

Nephrodium barbigerum T. Moore *ex* Hook., Sp. Fil. **4** : 113 (1862).

Habitat: Rare in forest around 3300m and above.

Dharamsala: Triund[6].

Stipes and rachis very densely scaly and fibrillose, scales pale or russet brown; lamina 2-pinnate, large, texture thickly herbaceous, both surfaces scaly, lower 2-3 pairs of pinnae gradually reduced in size.

***D. caroli-hopei* Fras.-Jenk.,** Bull. Br. Mus. nat. Hist. (Bot.) **18** (5): 422 (1989).

D. marginata sensu auct. West Himalaya, *non* (Wall. *ex* Clarke) Christ.

Habitat: Fairly common on the moist and humus rich forest floor or along road-sides between 1200 and 1800m or above.

Bir (SS-86), Billing (SS-201), Sansal (SS-771), Kandbari (SS-859); Dharamsala: Mcleodganj, Dull Lake[6].

Lamina large, broadly triangular-lanceolate, widely truncate at base; entire frond fertile.

***D. chrysocoma* (Christ) C. Chr.,** Index Fil. **1** : 257 (1905).

Aspidium filix-mas Sw. var. *chrysocoma* Christ, Bull. Herb. Boisss. **6** : 966 (1898).

Habitat: Occasional; inhabits humus rich forest-floor or rock-crevices or damp forest slopes between 1800 and 2700m.

Bir (SS-92), Multhan (SS-615), Kothi-Kohr (SS-503), Khaniyara (SS-307); Dharamsala: Dharamsala K.B., Mcleodganj[6].

Lamina 2-pinnate, base slightly truncate, turning yellowish in autumn; pinnules rounded truncate, apex rounded, margin crenate; entire frond fertile; indusia light-brown turning grey at maturity; sori large.

***D. cochleata* (Buch.-Ham. *ex* D. Don) C. Chr.,** Index Fil. **1** : 258 (1905).

Nephrodium cochleatum Buch.-Ham. *ex* D. Don, Prodr. Fl. Nepal : 6 (1925).

Lastrea cochleata (Buch.-Ham. *ex* D. Don) Moore, Index Fil. : 88 (1858).

Habitat: Rare on the forest-floor around 1800m and above.

Bir (SS-97); Dharamsala[6].

Fronds dimorphic with many spreading drooping sterile fronds and few (one or more) fertile fronds standing stiffly erect in the middle; sori occupy the entire lower surface of the fertile contracted lobe.

***D. juxtaposita* Christ,** Bull. Acad. int. Geogr. Bot. **17** : 138 (1907).

Lastrea odontoloma Moore, Index Fil. : 90 (1858), *nom. nud., non* Beddome (1864), *nec Dryopteris odontoloma* (Bedd.) C. Chr.

Dryopteris odontoloma auct. West Himalaya *non* Bedd.

Habitat: Fairly common on the humus rich forest-floor or even along road-sides between 1300 and 2000m.

Bir (SS-95), Billing (SS-215), Sansal (SS-777), Kandbari (SS-862); Dharamsala: Forsytheganj, Mcleodganj[6].

Stipes with brownish to jet black glossy scales at base; upper surface of lamina green or hardly blue-green, lower surface whitish green; pinnules rather small, some pinnules at base of pinnae may be curved on the basiscopic side; triploid apomict.

***D. nigropaleacea* (Fras.-Jenk.) Fras.-Jenk.,** Bolm. Soc. Broteriana II **55** : 238 (1982).

D. pallida (Bory) C. Chr., *apud.* Maire & Petitm. subsp. *nigropaleacea* Fras.-Jenk., Candollea **32** : 316(1977).

Habitat: Common in humus rich places on the forest-floor between 1200 and 2700m.

Bir (SS-131), Sansal (SS-796), Multhan (SS-626), Kothi-Kohr (SS-422), Khaniyara (SS-304), Gopalpur (SS-46), Satovari (SS-285); Dharamsala: Forsytheganj, Mcleodganj[6].

Scales at stipe base dark-brown or blackish; lamina lower surface pale-green, upper surface mid or blue-green, often glaucous green (especially in dried specimens), matt; pinnule margin lobed more or less into 4-6 pairs of rounded truncate sharply toothed lobes; diploid sexual.

***D. panda* (Clarke) Christ,** Bull. Acad. int. Geogr. Bot. **19** : 175 (1909).

Nephrodium filix-mas Rich. *var. panda* Clarke, Trans. Linn. Soc. London 2 Bot. **1** : 519 (1880).

Nephrodium panda (Clarke) Hope, J. Bomb. nat. Hist. Soc. **12** : 623 (1901).

Habitat: Rare amidst low bushes on open slopes or occasionally in the upper part of the forests at altitude above 2700m.

Dharamsala: Triund[6].

Lamina somewhat narrowly lanceolate with a truncate base; pinnae margin lobed 1/3 to 1/2 or slightly more to the costa; sori large, indusiate. A fern of high altitudes.

***D. ramosa* (Hope) C. Chr.,** Index Fil. **1** : 287 (1905).

Nephrodium ramosum Hope, J. Bot. (London) **34:** 126 (1896).

Habitat: Rare; inhabits forest-floor amongst rocks around 2400m and above.

Kothi-Kohr (SS-481).

Lamina deltate or widely triangular-lanceolate, widest at base; sori indusiate, small, round.

***D. redactopinnata* Basu & Panigr.,** Indian J. Forestry **3** : 270 (1980). (Plate 7)

Habitat: Rare; a "basket fern" of the forest-floor around 2700m.

Dharamsala[10].

Stipes short, very densely scaly; lamina pinnate, gradually tapering towards base, texture thinly herbaceous, both surfaces fairly fibrillose; lower 4-6 pairs of pinnae gradually reduced but not downwards deflexed; most veins forked. (Plate 8).

***D. serratodentata* (Bedd.) Hayata,** Icon. Pl. Formos. **4** : 179, t.116 (1914).

Lastrea filix-mas Pr. var. *serratodentata* Bedd., Handb. Ferns Brit. India Suppl.: 55 (1892).

Habitat: Rare in rock-crevices between 3000-4000m. Dharamsala[3].

Stipes thin, base sparsely scaly, scales pale-brown; rachis very sparsely scaly or almost glabrous; lamina 1-2 pinnate, small, texture thinly herbaceous, upper surface glabrous; lowest pinnae the largest or slightly smaller than the pair above.

***D. sparsa* (Buch.-Ham. *ex* D. Don) O. Ktze.,** Revis. Gen. Pl. **2** : 813 (1891).

Nephrodium sparsum Buch.-Ham. *ex* D. Don, Prodr. Fl. Nepal : 6 (1 825).

Habitat: Rare on forest floor around 1200m.

Bir (SS-112).

Lamina triangular lanceolate, widest at base, texture herbaceous; margin of pinnules serrate or lobed with an acute tooth above the sinus between the lobes. Sori medium-sized.

***D. stewartii* Fras.-Jenk.,** Nova Hedwigia **16:** 467 (1969) ["1968"].

D. odontoloma (Bedd.) C. Chr. forma *brevifolia* Mehra & Khullar, Res. Bull. (N.S.) Panjab Univ. **25:** 147 [1974] (1980) *nom. nud.*

Plate 7
Dryopteris redactopinnata
A. Portion of lamina magnified (x 3).

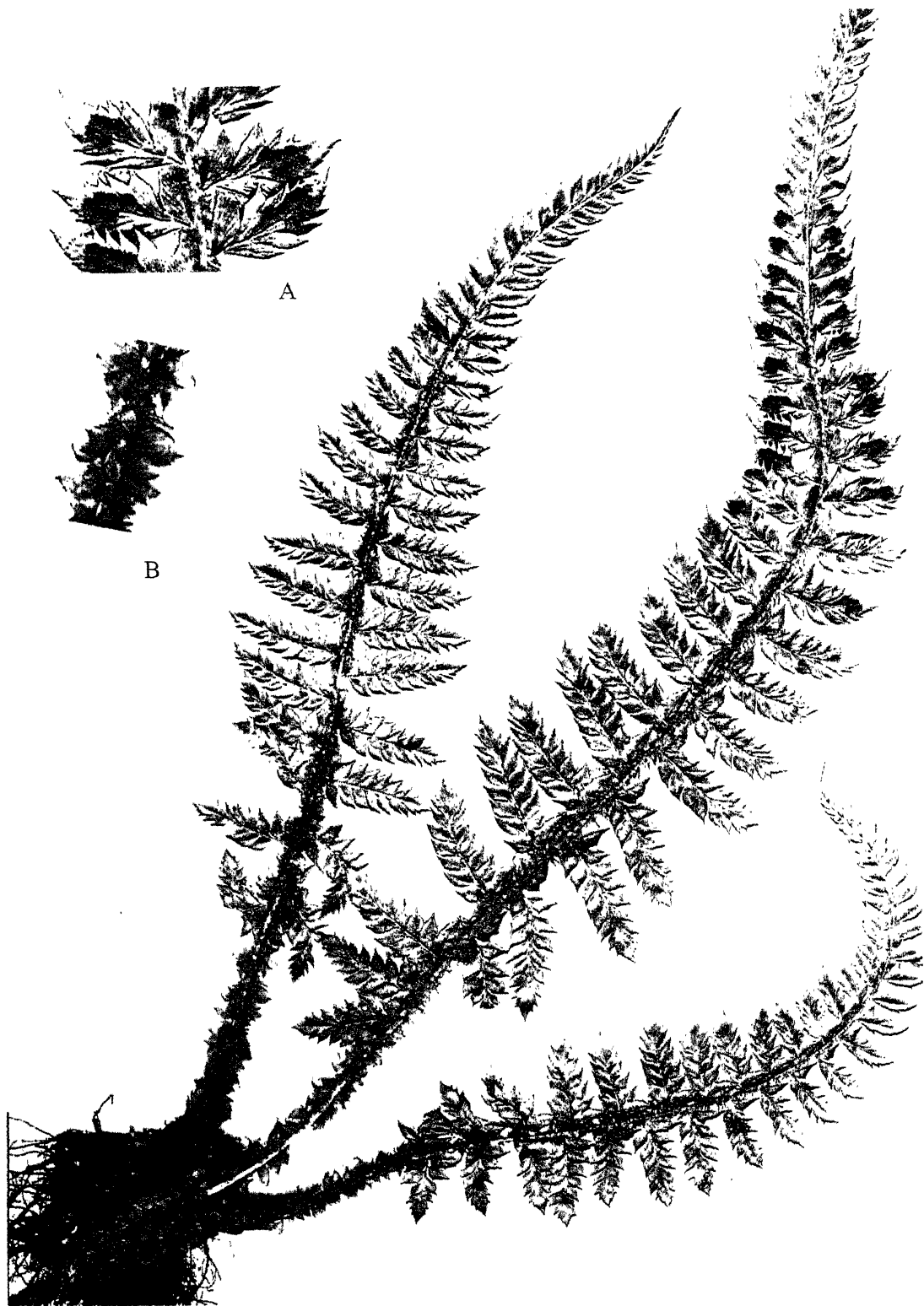



Plate 8
Polystichum mehrae
A. Part of lamina magnified.

Habitat: Occasional amongst rocks in the forest or on moist slopes around 2700m and above.

Multhan (SS-650).

Lamina widest at or just above the base, upper surface mid-green not bluish-green, lower surface green not whitish-green; pinnules oblong lanceolate, apex acute, bearing many small acute teeth, usually ending in one, sides in their upper halves sloping towards the apex, margin deeply lobed to 1/2 or 3/4 to the costule in large well developed pinnules towards the base of the lamina.

***D. subimpressa* Loyal,** Nova Hedwigia **16** : 467 (1969) ["1968"].

D. subodontoloma Loyal, in Mehra Res. Bull. (N.S.) Panjab Univ. **12** : 153 (1961) *nom. nud.*

Habitat: Very rare along water channels or streamlets in the forest around 2400m and above.

Kothi-Kohr(SS-410).

Stipes and rachis scaly; lamina 2 becoming 3-pinnate at base; basiscopic pinnules generally longer than the acroscopic ones only in the lowest pinnae, basiscopic basal pinnule in the lowest pair of pinnae the largest.

***D. x wechteriana* Fras.-Jenk.,** Bull. Br. Mus. nat. Hist. (Bot.) 18(5): 466 (1989).

(= *D. chrysocoma* x *D. nigropaleacea*)

Habitat: Rare on the moist forest-floor around 2500m.

Kangra district: Kothi-Kohr (SS-210).

Intermediate in morphology between its parents. It differs from *D. chrysocoma* in long stipes with wide pale scales; lamina wider at base; pinnules with shallow side lobes and prominent wide based teeth; indusia large, crowded, lifting up at maturity.

***D. woodsiisora* Hayata,** Icon. Pl. Formos. 6: 158 (1916).

Habitat: Rare along road-sides around 2700m.

Billing (SS-272).

Stipes stramineous, long but shorter than the lamina, sparsely scaly except at base where it is densely scaly, scales deciduous, leaving a scar upon falling; lamina lanceolate.

***D. xanthomelas* (Christ) C. Chr.,** Index Fil. Suppl. **2** : 41 (1913).

Aspidium xanthomelas Christ, Bull. Geogr. Bot. Mans.: 117 (1906).

Dryopteris pulcherrima Ching, Bull. Fan Mem. Inst. Biol. (Bot) **8** : 422 (1938).

D. fibrillosa (Clarke) Hand.-Mazz., Anz. Akad. (1905). Wiss. Wein **7** : 2 (1922), *non* (Bak.) C. Chr. (1905)

D. sinofibrillosa Ching, Bull. Fan Mem. Inst. Biol. (Bot.) **10** : 180 (1940).

Habitat: Uncommon on the forest - floor around 2700m.

Kothi-Kohr (SS-453); Dharamsala: Triund[3].

Stipes short with dense very dark-castaneous brown to more usually black, concolorous, shinning scales and fibrils; very few or none of the veins in a pinnule forked.

***POLYSTICHUM* Roth,** Tent. Fl. Germ. **3** : 31, 69 (1799) *nom. cons.*

***P. bakerianum* (Atkins. *ex* Clarke) Diels,** Nat. Pfl. **1** : 191 (1899).

Aspidium bakerianum Atkins. *ex* Clarke in Hook., Ic. pl. : t. 1656 (1886).

Habitat: Very rare in the forest above 3000m.

Dharamsala[3].

Lamina pinnate becoming nearly 2-pinnate, base broadly truncate, or much tapered below to auricles, texture subcoriaceous or herbaceous, upper surface scantly fibrillose or glabrous.

***P. discretum* (D. Don) J. Smith,** J. Bot. **3** : 413 (1841).

Aspidium discretum D. Don, Prodr. Fl. Nepal : 4 (1825).

Polystichum aculeatum sensu auct. India non (L.) Roth.

Polystichum nigropaleaceum (Christ) Diels, in Engl. & Prantl, Nat. Pfl. Fam. 1-4: 191 (1899).

Polystichum setiferum (Forssk.) Woynar var. *crenatum* Nair, Amer. Fern J. **64** : 15 (1974).

Habitat: Fairly common; inhabits humus rich forest-floor, sometimes the edges of open places between 1200 and 1800m.

Bir (SS-122), Sansal (SS-740), Kandbari (SS-808), Khaniyara (SS-339); Dharamsala: Khanjjar Mahadev Temple[6].

Stipes with light-deep brown to almost black linear lanceolate (never broad) scales; broad lanceolate scales on rachis absent; pinnules with an acutely pointed apex with prominent teeth, margin serrate-dentate to spinulosely serrate; diploid sexual.

***P. x jamunae* Fras.-Jenk.,** Aspects Pl. Sci. **13** : 278 (1991).

(=P. mehrae x P. squarrosum)

Habitat: Rare on the forest edges along road-side in humus rich conditions around 2000m.

Sansal (SS-182).

Similar to *P. mehrae,* but slightly larger and with straight fronds (thus approaching *P. squarrosum),* but the fronds are narrower and more compact than in that species. Spores abortive.

***P. lentum* (D. Don) Moore,** Index Fil. : 86 (1858).

Aspidium lentum D. Don, Prodr. Fl. Nepal : 4 (1825).

Habitat: Occasional along forest edges in deep sheltered and shaded conditions between 1200 and 2000m.

Sansal (SS-702).

Stipes rather long; lamina pinnate, with a characteristic subterminal proliferous bud; auricles at the base of the pinnae free, well developed and almost parallel to the rachis.

***P. mehrae* Fras.-Jenk. & Khullar,** Indian Fern J. **2** : 10 (1985). (Plate 8)

P. acanthophyllum sensu auct. Indo-Himalaya, *non* (Franch.) Christ.

Habitat: Occasional in rock-crevices around 2000m and above.

Bir (SS-150), Sansal (SS-714); Dharmsala: Triund[6].

Stipes short, densely scaly and fibrillose; lamina pinnate, characteristically never straight but curved sideways spreading out and pressed flat on the surface of rocks, texture very coriaceous, lower surface paler, scantily fibrillose, upper surface dark-green, glossy, glabrous. (Plate 12).

***P. nepalense* (Spr.) C. Chr.,** Index Fil. **1** : 84 (1905).

Aspidium nepalense Spr., Syst. Veg. **4** : 97 (1827).

Habitat: Rare along ravines or water falls in shade between 2100 and 3100m.

Dharamsala: Triund near Alwas[6].

Lamina pinnate, narrowly lanceolate; pinnae ovate-oblong, falcate, margin almost entire or closely dentate serrate, all around the margin is a scarious pale mucronately toothed fringe, lower pinnae slightly reduced in size compared to those above.

***P. obliquum* (D. Don) Moore,** Index Fil. : 87 (1858).

Aspidium obliqum D. Don, Prodr. Fl. Nepal : 3 (1825).

Habitat: Uncommon on dark, moist, dripping rocks in shade between 1200-2100m.

Sansal (SS-746), Lohardi (SS-970); Dharamsala: Triund[6].

Lamina pinnate, texture subcoriaceous, dull-green, not glossy; pinnae rhomboido-ovate to ovate oblong. The plants show subdimorphism, where the fertile fronds and their pinnae are generally smaller than the sterile ones, although this is not a fixed character and the two may be of the same size. Sterile fronds outnumber the fertile ones.

***P. piceopaleaceum* Tagawa,** Acta Phytotax. Geobot. **5** : 255 (1936).

Aspidium angulare sensu Hope, J. Bomb. nat. Hist. Soc. **14** : 471 (1902).

Polystichum setiferum sensu auct. India non (Forssk.) Moore *ex* Woynar.

Habitat: Common on the forest-floor or in moist places along the forest edges between 1500-2500m in the west Himalaya.

Bir (SS-149), Sansal (SS-760), Kandbari (SS-873), Kothi-Kohr (SS-515); Dharamsala: Triund[5]

Stipes with broad ovate lanceolate or ovate lanceolate scales with variable colour (dark-brown or

golden brown or bicolorous with a central dark region and paler at the margins); rachis also similarly clothed; lamina 2-pinnate; pinnae small; diploid sexual.

***P. shemiense* Christ,** Bull. Geogr. Bot. Mans. **16**: 113 (1906).

Habitat: Rare in drier inner ranges above 3000m. altitude.

Kangra[10].

Stipes rather short, scaly and fibrillose; lamina pinnate, narrow, herbaceous, lower surface scantily fibrillose, upper surface glabrous; pinnules small, apex with a single pointed tooth, fewer shorter teeth elsewhere on margin.

***P. squarrosum* (D. Don) Fee,** Mem. Fam. Foug. 5, Gen. Fil. : 278 (1850-52).

Aspidium squarrosum D. Don, Prodr. Fl. Nepal : 4 (1825).

Habitat: Very common on the forest-floor or along the edges of forest between 1200 and 3000m.

Bir (SS-162), Billing (SS-278), Sansal (SS-764), Kandbari (SS-805), Multhan (SS-616), Kothi - Kohr (SS-517), Khaniyara (SS-327); Dharamsala: Mcleodganj[5].

Stipes and rachis densely scaly and fibrillose; lamina texture coriaceous, stiff, heavy, hard, greyish-green, upper surface glabrous, glossy.

***P. stimulans* (Kunze *ex* Mett.) Bedd.,** Ferns Brit. India: t. 31 (1865).

Aspidium stimulans Kunze *ex* Mett., Farng. *Pheg. Asp,:* 327 (1858).

Habitat: Rare; inhabits shaded moist rock-crevices between 2200 and 2800m.

Dharamsala: Triund[6].

Stipes thin, fragile, scaly and fibrillose; lamina pinnate, straight; both surfaces glabrous; fronds hang from rock crevices.

***P. yunnanense* Christ,** Notul Syst. [Paris] **1** : 34 (1909).

Habitat: Uncommon in the humus rich habitats on the forest-floor around 2000m and above.

Bir (SS-151), Khaniyara (SS-390).

Stipe base with wide ovate scales which do not normally extend far up the stipe before becoming small and are usually not as dark as in *P. piceopaleaceum;* lamina often dark bluish-green, with slight depressions on the upper surface of the lamina above each sorus; pinnules elongated, margin more lobed and apices more acute than in *P. piceopaleaceum;* lowest acroscopic pinnule on each pinna frequently long; a tetraploid sexual.

TECTARIACEAE

***TECTARIA* Cav.,** Anales Hist. Nat. **1(2)** : 115 (1799).

***T. coadunata* (J. Sm.) C. Chr.,** Contrib. U.S. Nation. Herb. **26** : 331 (1931).

Sagenia coadunata J. Sm., in Hook. J. Bot. **4** : 184 (1862) *pro parte.*

Tectaria macrodonta (Fee) C. Chr., Index Fil. Suppl. **3** : 181 (1934) *nom. superfl.*

Habitat: Rare amongst rocks, in rock-crevices, in cliffs next to water-seepage between 1200 to 1700m.

Dharamsala: Dharamsala K.B.[7].

Lamina 1-3 pinnate (in large fronds), upper surface pilose and softly hairy, lower glabrous; generally the lamina has 1-3 pairs of free pinnae followed by a broadly adnate pinnatifid distal part; veins anastomosing to form areolae.

ASPLENIACEAE

***ASPLENUM* L.** Sp. pl. **2** : 1078 (1753).

***A. dalhousiae* Hook.,** Ic. pl.: t.105 (1837).

Habitat: Very common; inhabits moist walls/slopes or rock crevices between 700 and 1800m.

Bir (SS-148), Billing (SS-256), Sansal (SS-703), Palampur (SS-45), Baijnath (SS-33);

Dharamsala: Forsytheganj[8].

Stipes and rachis scaly; lamina simple, margin deeply pinnatifid to variously lobed at times approaching a pinnate condition; lobes triangular oblong or ovate, alternate from one side to the other, apex obtuse, margin almost entire or crenately lobed.

***A. indicum* Sledge,** Bull. Br. Mus. nat. Hist. (Bot.) **3** : 264 (1965). (Plate 9)

Habitat: Rare; an epiphyte/lithophyte on shaded rocks between 1800-2000m.

Dharamsala: Forsytheganj[8].

Lamina pinnate; pinnae dimidiate-ovate, acute, upper base broadly cuneate, sub-auriculate, lower base narrowly cuneate and entire to half or more the length from base, remaining margin of pinnae on both sides irregularly shallowly lobed with teeth-like projections, lower pinnae not much reduced or the lower pair slightly smaller than those above. (Plate 2).

A. laciniatum **D. Don,** Prodr. Fl. Nepal : 8 (1825).

A. varians Wall. *ex* Hook. & Grev., Ic. Fil. **2** : t 172 (1829), *nom. superfl.*

Habitat: Uncommon on moist shaded rocks, forest edges or even at the base of tree trunks between 1800-2000m. (Plate 10).

Bir(SS-124).

Lamina 2-pinnate, finely dissected, widest below the middle; the lowest pair of pinnae usually a little shorter than the second pair, which may be of the same size as or a little longer or at times as long as the third or fourth pairs; tetraploid sexual.

A. tenuicaule **Hayata,** Icon. Pl. Formos. **4** : 228 (1914).

A. subvarians Ching, in C. Chr. Index Fil. **3** : 38 (1934).

Habitat: Occasional on moist shaded rocks, forest edges/slopes around 2000m.

Lohardi (SS-986).

Lamina 2-pinnate; ultimate lobes rounded ovate; pinnules borne on long thin delicate stalks, lowest pair of pinnae smaller than the second pair, the third generally the largest; diploid sexual.

A. trichomanes **L.,** Sp. pl. **2** : 1080 (1753).

Habitat: Uncommon; inhabits crevices of rocks in shade or moist forest slopes around 2000m and above.

Kothi-Kohr (SS-505); Dharamsala: Bhagshu Nath, Triund[8].

Lamina pinnate; pinnae orbicular or sublong, base cuneate, margin finely crenate serrate.

DAVALLIACEAE

ARAIOSTEGIA **Copel.,** Philipp. J. Sci. **34** : 240 (1927).

A. delavayi **(Bedd. *ex* Clarke & Bak.) Ching,** Fl. Reip. Pop. Sin. **2** : 289 (1959).

Davallia pulchra D. Don var. *delavayi* Bedd. *ex* Clarke & Bak., J. Linn. Soc. **2** : 410 (1886).

Habitat: A rare epiphyte between 2700 and 3000m.

Multhan (SS-638); Dharamsala: Triund[7].

Scales on rhizome overlapping, broad, ovate, margin entire, apex acute; stipes short, 1/3 or 1/4 the length of lamina; lower 1 or 2 pairs of lower pinnae gradually shortened.

A. pseudocystopteris **(Kunze) Copel.,** Philipp. J. Sci. **34** : 241 (1927).

Leucostegia pseudocystopteris Kunze, Bot. Zeit: 68 (1850).

Davallia pulchra D. Don var. *pseudocystopteris* (Kunze) Clarke, Trans. Linn. Soc. London 2 Bot. **1** : 444 (1880).

Habitat: A very common epiphyte on tree-trunks and branches or on moist, shaded walls/rocks around 1200m and above.

Bir (SS-59), Billing (SS-253), Sansal (SS-741), Multhan (SS-637), Kothi-Kohr (SS-452), Khaniyara (SS-342); Dharamsala: Mcleodganj[7].

Scales on rhizome adpressed, brown, bicolorous; stipes almost as long as or shorter than the lamina, a few sparse deciduous adpressed scales on stipe. (Plate 11).

BLECHNACEAE

WOODWARDIA **J. Sm.,** Mem. Acad. Sc. Turin **5** : 411 t 9(3), (1793).

W. unigemmata **(Mak.) Nakai,** Bot, Mag. Tokyo **39** : 103 (1925).

W. radicans (L.) J. Sm. var. *unigemmata* Makino, J. Jap. Bot. **2** : 7 (1908).

Habitat: Fairly common in wet localities, along road-sides or in the forest between 1300 and 2500m altitude.

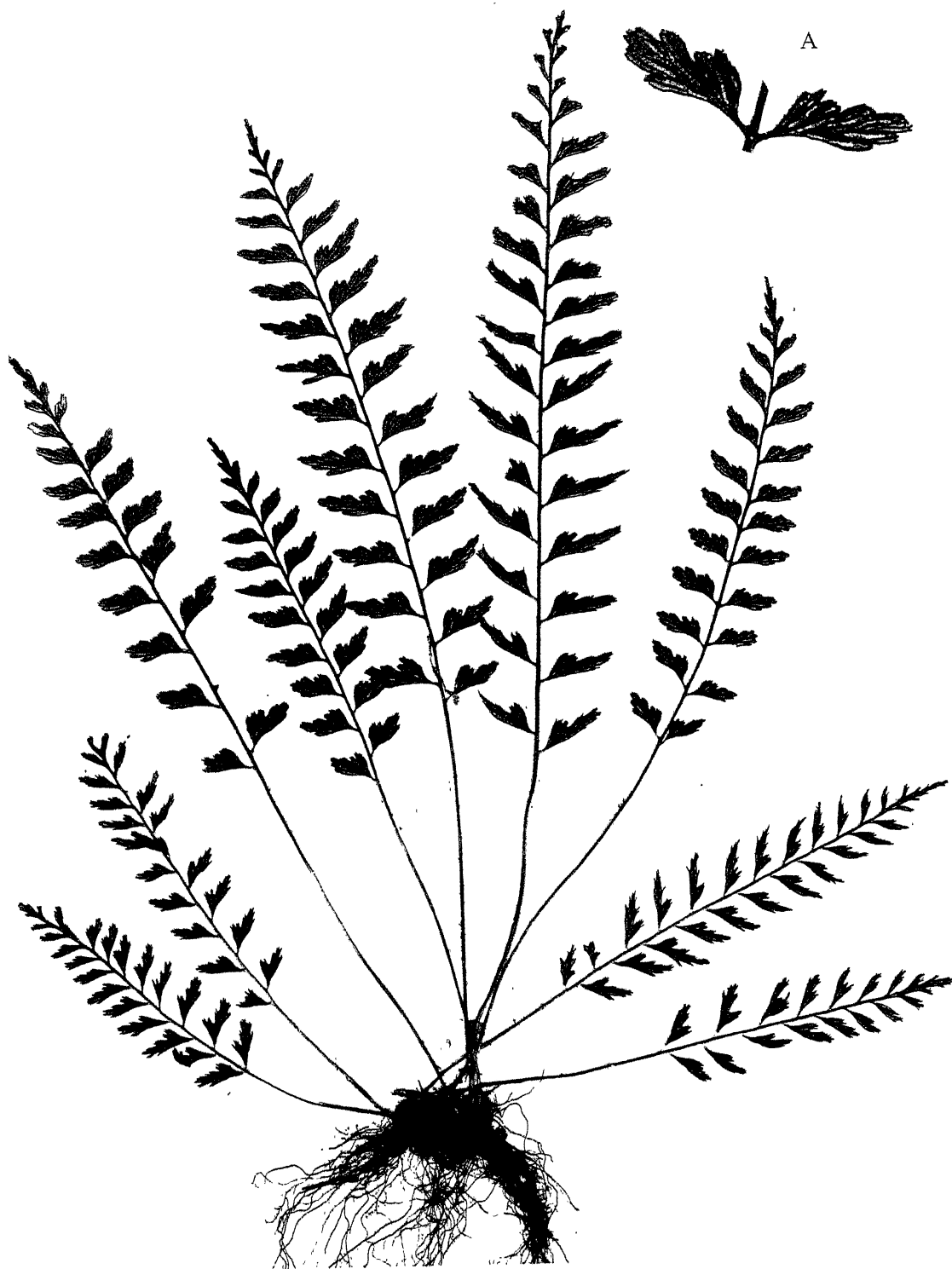



Plate 9
Asplenium indicum
A. Two pinnae magnified (x 5).

Plate 10
Asplenium laciniatum
A. Part of lamina magnified (x 5).

Plate 11
Araiostegia pseudocystopteris
A. A portion magnified (x 3).

Plate 12
Lepisorus mehrae
A. Portion part of lamina magnified (x 3).

Bir (SS-195), Multhan (SS-611), Andreta (SS-54); Dharamsala: Dharamkot[8].

Frond large to huge; lamina pinnate, texture subcoriaceous, a subterminal proliferous bud present; veins anastomosing to form a costal and two or three costular areolae, free towards the margin; sori rectangular, deeply sunk in a cavity with raised margins, in a single row on either side close and parallel to the costae.

POLYPODIACEAE

***LEPISORUS* (J. Sm.) Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) **4** : 56 (1933).

***L. mehrae* Fras.-Jenk.,** New Species Syndrome Indian Pteridology Ferns Nepal : 159 (1997).

Polypodium kashyapii Mehra, Ferns Mussoorie, Punjab Univ. Publ., Lahore: 24 (1939) *nom. nud.*

Pleopeltis kashyapii Mehra *ex* Alston & Bonner, Candollea **15** : 208 (1956) *nom. nud.*

Lepisorus kashyapii (Mehra) Mehra, Res. Bull.(N.S.) Panjab Univ. 15(1 & II): 168 (1962) *nom. nud.*

Habitat: An uncommon epiphyte/lithophyte around 2400m.

Bir (SS-61); Dharamsala: Forsytheganj, Mcleodganj[9].

Stipes usually crowded towards the growing tip of the rhizome forming a 'basket-like' structure; fronds turn bright-brown upon drying; lamina thick coriaceous; veins obscure. (Plate 12).

***L. nudus* (Hook.) Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) **4** : 83 (1933).

Pleopeltis nuda Hook., Exot. Fl. Pl: 63 (1823).

Habitat: Common epiphyte/lithophyte around 2000m.

Sansal (SS-715), Multhan (SS-684); Dharamsala: Chamunda Devi Temple[9].

Rhizome scales dark-brown, concolorous, luminae uniform clear isodiametric, base broad, margin toothed, apex acuminate or acute, often loosing the apex when the scales are old.

***L. pseudonudus* Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) **4** : 83 (1983).

L.jakonensis (Blanf.) Ching, Acta Botanica Yunnanica **5(1)** : 5 (1983).

L. pseudoclathratus Ching & S.K. Wu, Acta Botanica Yunnanica **5(1)** : 10 (1983).

Habitat: A rare epiphyte/lithophyte between 1800-3000m.

Dharamsala: Triund[10].

Scales on rhizome with long filamentous teeth-like projections, apex long acuminate, luminae not clear; lamina tape-like; sori round or oblong, sometimes confluent.

***L. sesquipedalis* Fras.-Jenk.,** Bot. Helv. **102**(2) : 153 (1992) *non* (Wall, *ex* J. Sm.) Fras.-Jenk., Pak. Syst 5(1.2) : 91 (1991) [1992].

Drynaria sesquipedalis J. Sm., Bot. Mag. 72 Comped.:13 (1846).

Lepisorus excavatus auct. India non (Bory) Ching [and vars. *himalayensis* Bir & Trikha; and var. *mortonianus* Bir & Trikha].

L. scolopendrium (Ching) Mehra & Bir, Res. Bull. (N.S.) Panjab Univ.**15**(I&II): 168 (1964).

Habitat: Very common epiphyte/lithophyte between 1400 and 2400m.

Bir (SS-105), Billing (SS-218), Sansal (SS-707), Kandbari (SS-836), Multhan (SS-642), Kothi-Kohr (SS-425), Khaniyara (SS-310); Dharamsala: Dharamsala K.B., Mcleodganj[9].

Scales on rhizome dark-brown, concolorous, margin slightly erosed, apex acuminate, luminae clear; lamina simple, does not turn brown upon drying.

***L. tenuipes* Ching & Khullar,** Indian Fern J. **1**: 91 (1984).

Habitat: An uncommon epiphyte/lithophyte between 2000 and 2400m.

Multhan (SS-678); Dharamsala[9].

Scales on rhizome very dark-brown, base broad, rounded, bicolorous with a central band and a very narrow band of light-brown marginal cells, margin toothed with very short teeth-like projections, apex acute; lamina base decurrent on stipe.

MICROSORUM **Link,** Hort. Reg. Bot. Berol. **2** : 110 (1833).

M. membranaceum **(D. Don) Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) **4** : 304 (1933).

Polypodium membranaceum D. Don, Prodr. Fl. Nepal : 2 (1825).

Habitat: Very common lithophyte on wet rocks along banks of streams or besides water-falls in forest, or even as an epiphyte between 1000 and 1700m.

Bir (SS-101), Sansal (SS-751), Khaniyara (SS-385); Dharamsala K.B., Khanjjar Mahadev temple[9].

Lamina simple, broad, thin membranaceous; sori scattered, usually in 2-5 parallel or irregular rows between a pair of lateral veins and 6-8 between margin and rachis.

M. zippelii **(Blume) Ching,** Bull. Fan Mem. Inst. Biol. (Bot.) **4** : 308 (1933).

Polypodium zippelli Blume, Fl. Jav. : 172 t 80 (1829).

Habitat: A very rare epiphyte/lithophyte around 1600m.

Bir(SS-177).

Lamina thickly chartaceous, apex acuminate; sori larger than in the species above, in a row between a pair of lateral veinlets and 4-6 between margin and rachis.

PHYMATOPTERIS **Pich. Ser.,** Webbia **28** : 460 (1973).

P. oxyloba **(Wall. *ex* Kunze) Pich. Ser.,** Webbia **28**: 464 (1973).

Polypodium oxylobum Wall. *ex* Kunze, Linnaea **24**: 255 (1851).

Polypodium trifidum D. Don, Prodr. Fl. Nepal : 3 (1825) *non* Hoffm.

Habitat: An occasional epiphyte or lithophyte, also on humus rich shaded walls around 2300m altitude.

Kothi-Kohr (SS-510); Dharamsala: Mcleodganj, Dull Lake[9].

Stipes never longer than the lamina, texture subcoriaceous; lobes of lamina with entire margin.

POLYPODIODES **Ching,** Acta Phytotaxa. Sinica **16** (4) : 26 (1978).

P. amoena **(Wall. *ex* Mett.) Ching,** Acta Phytotax. Sinica **16(4)** : 27 (1978).

Polypodium amoenum Wall. *ex* Mett., in Abhn. Senck. Natur. Forh. Ges. **2** : 80 (1857).

Goniophlebium amoenum (Wall. *ex* Mett.) J. Sm., in Hook. Gen. Fil. ad. t 51 (1840).

Habitat: Fairly common as an epiphyte/lithophyte around 1800m and above altitude.

Bir (SS-77), Sansal (SS-721), Kandbari (SS-817), Kothi-Kohr (SS-404); Dharamsala: Mcleodganj[9].

Plants robust; lamina pinnate, texture thick, herbaceous to subcoriaceous; lower surface of rachis scaly throughout; pinnae lobes many.

P. lachnopus **(Wall. *ex* Hook.) Ching,** Acta Phytotax. Sinica **16** : 27 (1978).

Polypodium lachnopus Wall. *ex* Hook., Ic. pl.: t 952 (1854).

Habitat: Common as an epiphyte/lithophyte around 1600m and above.

Bir (SS-75), Sansal (SS-792); Dharamsala: Dharamsala K.B, Forsytheganj, Mcleodganj[9].

The rhizome of this delicate and pretty fern creeps (never buried) over the surface of rocks; scales on rhizome blackish, iridescent.

P. microhizoma **(Clarke *ex* Bak.) Ching,** Acta Phytotax. Sinica **16** : 27 (1978).

Polypodium microrhizoma Clarke *ex* Bak., in Hook. & Bak. Syn. Fil. : 51 (1871).

Goniophlebium microrhizoma (Clarke *ex* Bak.) Bedd., Ferns Brit. India Suppl.: 21 t. 384 (1876).

Habitat: An occasional epiphyte/lithophyte around 1500m and above.

Bir (SS-70), Sansal (SS-717), Kandbari (SS-822); Dharamsala: Forsytheganj, Mcleodganj, Dull lake[9].

Rachis generally glabrous, pinnae lobes between 20-25 pairs; veins anastomosing to form a single series of costal areolae, but free towards apex of lobe, costal

arches of the main rachis usually broken at least in the lower half of the lamina.

***PYRROSIA* Mirbel,** Hist. Nat.Gen. **4** : 70 (1803).

***P. flocculosa* (D. Don) Ching,** Bull. Chinese Bot. Soc. **1** : 66 (1935).

Polypodium flocculosum D. Don, Prodr. Fl. Nepal: 1 (1825).

Habitat: Very common epiphyte/lithophyte between 1200 and 1800m.

Bir (SS-108), Billing (SS-250), Sansal (SS-745), Satovari (SS-335), Mcleodganj, Dharamsala K.B.[9].

Lamina simple, base rounded or unequally hastate, one-half ending before the other or both halves decurrent on stipe; lower surface brown or grayish white, densely hairy.

***P. stictica* (Kunze) Holtt.,** Novit. Bot. Inst. Bot. Carol. Prag. **1968** : 30 (1969).

Niphobolus sticticus Kunze, Linnaea **24** : 257 (1851).

Habitat: Occasional epiphyte/lithophyte around 2400m.

Multhan (SS-624).

Scales on rhizome lanceolate with long marginal filaments; lamina simple, lanceolate or oblanceolate or linear lanceolate, base gradually narrowed and extending to the very base of stipe; lamina lower surface brown, hairy, upper surface glossy at maturity.

The Himalaya support *c.*700 species of Pteridophytes, of which 325 are from the west Himalaya. Himachal Pradesh has approximately 250 species with most of these in the mid-altitude zone (1500-3000m altitude). The number of natural sterile hybrids here is nine (excluding allopolyploids). The district of Shimla has the maximum number of 188 species. The Kangra district with 130 species is the fourth Pteridophyte rich district of Himachal Pradesh (Jatinder Chadha, 2006, Ph.D. thesis, Panjab University, Chandigarh). In Kangra, the genera *Athyrium* and *Dryopteris* have 16 species each followed by *Thelypteris* (14) and *Polystichun* (13). From there on a huge reduction of species is noted with *Cheilanthes* and *Lepisorus* with 6 each, *Adiantum* and *Pteris* 5 each, and *Asplenium, Coniogramme* and *Deparia* with 4 species each. Eighteen genera are represented by only a single species. The district of Kangra is quite unique in the distribution of species of Pteridophytes. While some species are quite uncommon or rare in other parts of the west Himalaya, these are quite common here. A few such examples are *Deparia japonica, Pyrrosia flocculosa* and *Thelypteris repens.* On the other hand some species that are fairly common elsewhere in the west Himalaya are rare here. *Hypodematium crenatum, Adiantum edgeworthii, Dryopteris cochleata, Asplenium laciniatum* and *Cyrtomium caryotideum* are some such species. A few species reported from a single locality earlier from Himachal Pradesh have been found in Kangra district. These species are *Dryopteris sparsa, D. subimpressa, D. x wechteriana, D. woodsiisora, Dennestaedtia scabra, Microsorum zippelii* and *Polystichum x jamunae.* In the above list the species mentioned as rare can be considered as threatened/ endangered. In Kangra district 45 species of Pteridophytes are rare; 22 are uncommon; 17 are occasional; 20 are very common, 14 are common and 12 are fairly common. Twelve species occur between 400-1000m altitude, 8 between 1000-1500m altitudes, 27 between 1500-1800m altitude, 21 between 1800-2200m altitude, 30 between 2200-2500m altitude and only 6 above 3000m altitudes. The numbers of epiphytes collected are only six. In addition 3 natural hybrids (determined on the basis of a large percentage of abortive spores and an intermediate morphology between the two suspected parents) have also been collected.

A number of suggestions for the conservation of Pteridophytes have been made from time to time. The dangers and threats to these plants have also been high-lighted which need not be repeated here. But the gravest threat is due to the ever increasing human population of the country. The bounties of nature have come under a lot of stress and pressure. This is due to the need of man rather than his greed. Once a human has come into this world he has to be clothed, fed and housed. For this, cutting of forests is the easy way out resulting in the destruction of the habitat of Pteridophytes. However, these days the most dreaded threat to ferns is the florist and those selling bouquets *etc.* Truck loads of fern fronds are collected from the wild and sold in the markets in the plains. If this ruthless, senseless and merciless massacre goes on unabated the day is not far off when even common ferns like *Polystichum squarrosum, P. piceopaleaceum, P. discretion, Dryopteris caroli-hopei, D. juxtaposita, D. nigropaleacea etc.* will become extinct. It appears the

beauty of the ferns has become their greatest enemy and a curse for these beautiful creations of nature. Urgent steps are required to stop their unwanted and uncontrolled exploitation as has been done for animals and trees. (Hunting and felling of trees has been banned in our country!). *In-situ* and *ex-situ* methods for their conservation and protection are a crying need of the hour. We must take every opportunity to correct and assist those in power to put an end to this destruction of ferns as this serves no useful purpose except to earn a few rupees and 'ape' the western culture. "To say it with Flowers". But the Western people cultivate those plants which are used for such purposes, while we simply destroy 'the goose that lays the golden egg' since we are too lazy to grow/cultivate them and believe only in the easy way out to earn something without any effort to replace what we are taking from nature.

Acknowledgements

Thanks to the Ministry of Environment & Forests, Government of India, for the AICOPTAX scheme sanctioned to the first author (SPK) which enabled us to undertake and complete this work. We are also grateful to Mr. C.R. Fraser-Jenkins for providing some information and also for his help in the identification of some specimens. One of us (SS) would also like to thank the C.S.I.R., New Delhi, for some financial assistance.

References

1. Desmond R. The European discovery of the Indian Flora. Royal Botanic Gardens, New York: Oxford University Press, 1992.

2. Beddome RH. Handbook to the Ferns of British India, Ceylon and the Malay Peninsula. Calcutta : Thacker Spink and Co, 1883.

3. Clarke CB. A review of the ferns of Northern India. *Trans Linn Soc London 2 Bot* 1880; **1** : 425-611.

4. Hope CW. The ferns of North-Western India including Afghanistan, the Trans-Indus protected states and Kashmir. *J. Bombay, nat Hist. Soc.* **12 (2)**: 315-325 (1899); **12 (3)**: 527-538 (1899); **12 (4)**: 621-633 (1899); **13 (1)**: 25-36 (1900); **13 (2)**: 236-251 (1900); **13 (3)**: 443-461 (1901); **13 (4)**: 657-671 (1901); **14 (1)**: 118-127 (1902); **14 (2)**: 252-266 (1902); **14 (3)**: 458-480 (1902); **14 (4)**: 720-749 (1903); **15 (1)**; 78-111 (1903); **15 (3)**: 415-429 (1904).

5. Schelpe EACLE. Ecological observations on Pteridophyta in the Kangra Himalaya. *Amer Fern J* 1954; **44** : 49-65.

6. Dhir KK, Datta KS. Ferns of Dharamsala Hills-2 (North Western Himalayas). Families: Woodsiaceae, Aspidiaceae, Athyriaceae. *Nova Hedwigia* 1976; **27** : 393-424.

7. Dhir KK, Datta KS. Ferns of Dharamsala Hills-1. (North Western Himalayas). Ophioglossaceaous and Schizeaeceous series. *J. Bombay nat. Hist. Soc.* 1977; **74** : 459-480.

8. Dhir KK, Datta KS. Ferns of Dharamsala-3. (North Western Himalayas). Families: Thelypteridaceae, Aspleniaceae and Blechnaceae. *Nova Hedwigia* 1977; **28** : 137-154.

9. Dhir KK, Datta KS. Ferns of Dharamsala Hills-4. (North Western Himalayas). Family: Polypodiaceae. *Nova Hedwigia* 1979; **29** : 105-120.

10. Khullar SP. An Illustrated Fern Flora of the West Himalaya. Vol. I & II. Dehra Dun, India. International Book Distributors, Vol. I 1994, Vol. I 2000.

11. Chandra S. The Ferns of India (Enumeration, Synonyms and Distribution). Dehra Dun International Book Distributors, 2000; 1-459.

12. Khullar SP. Pteridophytes-Nature's failed Experiment. In: Prasher IB, Ahluwalia As, editors. Plant Taxonomy-An overview. Chandigarh: Department of Botany, Punjab University, 2006.

13. Sharma S, Khullar SP. Fern Hybrids from Kangra Himalaya. *J Indian Bot Soc* 2004; **83** : 54-60.

14. Fraser-Jenkins CR. The fern and allies of the Far West Himalaya: Some additions and corrections. *Bot Helv.* 1992; **102** : 143-157.

15. Fraser-Jenkins CR. New Species Syndrome in Indian Pteridology and the Ferns of Nepal. Dehra Dun : International Book Distributors, 1997.

Proc. Nat. Acad. Sci. India, Sect. B, Vol. 78 Pt. I, 2008

Free redical scavenging and nitric oxide synthase activation in murine lymphocytes and ehlrich ascitic carcinoma cells treated with ethanolic extract of tumeric

A.K. CHAKRAVARTY* and HADIDA YASMIN

Immunology and Cell Biology Laboratory, Dept. of Zoology, University of North Bengal, Siliguri-734430 (WB), India.

**e-mail: prof_ashim_chakravarty@rediffmail.com*

Received October 27, 2006, Revised June 27, 2007, Accepted October 25, 2007

Abstract

Ethanolic turmeric extract (ETE) leads to inhibition and scavenging of strong oxidant, such as superoxide (O_2^-), hydrogen peroxide ($H_2O_2^-$) and hydroxyl radical (OH^-), both in case of lymphocytes as well as tumor cells. At the same time ethanolic turmeric extract was found to stimulate the lymphocytes to increase nitric oxide (NO) production probably for mounting cytotoxic response towards tumor cells by activating the L- arginine derived nitric oxide synthase (NOS) pathway. Increase in the nitric oxide in tumor cells seems to set apoptosis in the cells which was further confirmed by scanning electron microscopy and light microscopy where the tumor cells showed cytoplasmic blebbing and multiple apoptotic body formation upon turmeric treatment. Whereas, the lymphocytes with turmeric treatment seemed to be quite healthy under SEM.

Keywords : turmeric, free radicals, lymphocytes, activation, antitumor.

सारांश

अल्कोहल युक्त हल्दी का सार, श्वेत रक्त कोशिकाओं तथा रसौली कोशिकाओं में सुपर आक्साइड (O_2^-), हाइड्रोजन पर आक्साइड ($H_2O_2^-$) तथा हाइड्राक्सिल रेडिकल (OH^-) जैसे शक्तिशाली आक्सीडेंट का प्रावरोध करता है। साथ ही साथ ऐसा सार श्वेत रक्त कोशिकाओं को नाइट्रिक आक्साइड (NO) बनाने को उत्प्रेरित करता है, जिससे L–आर्जनीन उत्प्रेरित नाइट्रिक आक्साइड सिन्थेज़ (NOS) क्रियाशील हो जाता है। इस कारण रसौली कोशिकाओं में स्वतः सृजन की प्रक्रिया दिखने लगती है। इसकी पुष्टि स्कैनिंग एलेक्ट्रान माइक्रोस्कोप तथा सामान्य माइक्रोस्कोप द्वारा कोशिका द्रव्य में अनेक एपोप्टोटिक संरचनाओं के द्वारा की जा सकती है। हल्दी के सार से प्रतिपादित श्वेत रक्त कोशिकायें SEM के माध्यम से स्वस्थ तथा निरोग दिखती है।

सांकेतिक शब्द : हल्दी, स्वतंत्र-आयन, श्वेत रक्त कण, उत्प्रेरण, रसौली प्रतिरोधी।

Introduction

Generation of free radicals by univalent reduction of O_2 are fundamental to any biochemical process and represents an essential part of the cellular metabolism.[1] There is a dynamic balance between the amount of free radicals generated in the body and cellular antioxidants to quench them and protect the body against their deleterious effects[2]. So, any additional burden of free radicals can tip the pro-oxidant and anti-oxidant balance leading to oxidative stress which certainly has negative cytopathologic consequences[3-7].

The unregulated and prolonged production of reactive oxygen species (ROS) in the form of superoxide (O_2^-), hydrogen peroxide ($H_2O_2^-$) and hydroxyl radical (OH^-) during the metabolism of certain chemical carcinogens has been linked to mutation[1,7-8] (oxidant-induced DNA damage), as well as modification of gene expression[9-10]. ROS induce cell proliferation during the tumor promotion stage of carcinogenesis[7]. Cellular receptors for growth modulator molecules are also affected by reactive oxygen species. The oxidizing molecule binds and activates epidermal and platelate derived growth factors and can activate downstream signaling cascade, which may contribute to carcinogenesis.[11]. In the signaling pathways, oxidants mostly affect mitogen-activated protein (MAP) kinases/AP-1 and NF-κB.[11-13]. The major pathways for cell signaling, which involve protein phosphorylation

and redox dynamic fluctuations, may have a colossal impact on cellular functions ranging from proliferation and differentiation to regulation of cell cycle events, apoptosis and under extreme conditions, necrosis[14-16]. Oxygen species therefore are important determinants of redox state and can interfere with the cells homeostasis and may lead to various pathophysiologic conditions.

Natural phenolic antioxidants from medicinal or edible plants have recently received much attention as promising agents for reducing the deleterious effects of oxidative stress-induced diseases[2,17]. Curcumin present in turmeric is an active phenolic compound which scavenges hydroxyl and superoxide anions[18-19]. Its antioxidant property, has further been shown by its capacity to inhibit lipid peroxidation in rat brain homogenate[20], in mouse red blood cells[21], in rat liver[22] and also in renal epithelial cell[23]. Curcumin also inhibits induction of iNOS in macrophages activated with lipopolysacharides and IFN-γ[24]. Besides its antioxidant and anti-inflammatory properties, curcumin has also been found to possess anti-infectious[25], anti-carcinogenic[26] and anti-tumor activity[27-29]. According to Lu *et al.* the anti-proliferative affectivity of curcumin to tumor cells is due to its ability to inhibit expressions of several proto-oncogenes; c-Jun and c-Fos in JB6 cells in mouse epidermis[30]. Khar *et al.* suggested that curcumin inhibited AK5 tumor growth and induced apoptosis through the activation of caspase-3[31].

Research works that have been carried out with turmeric till date are mostly to show its inhibitory property towards tumor growth[2729,31], carcinogens[26], mutagens[30] and viruses[25]. The present investigators analyzed the effect of turmeric on immunocompetent cells as this was not much looked into[28-29]. This is important in view of significant antitumor response of T cells. In the present investigation, the status of generation of free radicals [such as superoxide (O_2^-), hydrogen peroxide ($H_2O^-_2$) and hydroxyl radical (OH^-) and nitric oxide (NO)], by lymphocytes and tumor cells in presence of ethanolic turmeric extract (ETE) has been studied. Earlier results of author showed 25 µl dose of ETE to be conducive for lymphocyte growth and simultaneously induced apoptosis in tumor cells[29]. The same has been used for the study of free radicals in the present investigation. Scanning electron microscopy has been included here to ascertain the condition of lymphocytes and tumor cells treated with turmeric. Generation of free radicals and their effects in lymphocytes and tumor cells might suggest certain immunotherapeutic approaches of turmeric against malignancy.

Material and Methods

Tumor Induction : Inbred adult Swiss mice of both sexes, 8-14 weeks of age, were used for all experiments. Ehlrich ascitic carcinoma cell line was obtained from Chittaranjan National Cancer Research Institute, Kolkata and maintained in our laboratory by serial passages. To continue with the cell line, adult mice were injected intraperitoneally with 10^6 ascitic fibrosarcoma cells per mouse in 0.1ml PBS for induction of ascitic tumor. Cell suspension was prepared in phosphate buffered saline (PBS) following standard protocol. Within 10 to 15 days full grown ascitic tumor develops. Serial passage was carried out after every 20 days. Average life span of ascitic tumor bearing mice is 28 ± 4 days.

Ethanol Turmeric Extract Preparation : Fresh rhizomes of turmeric (*Curcuma longa* Linn.,) were obtained from local market. After cleaning properly with water; 10 g of sample was crushed to a paste with morter and pestle and 10 ml of absolute alcohol was added to this paste and kept in a refrigerator at 4°C. After 12 h the alcoholic suspension of turmeric was taken out and filtered through Whatman filter paper 1; the filtrate was refiltered again through cellulose acetate Millipore filter paper (0.2µm porosity, Sartorius) for sterilization, and the final solution obtained was stored at 4°C for further use. The yield of turmeric in ethanolic extract (ETE) was 0.435 ± 0.032 mg/ml.

As the turmeric extract was made in ethyl alcohol, the equivalent amount of ethanol (25µl) was used for control, and this protocol was maintained for all the experiments.

Superoxide scavenging assay : Superoxide radical (O_2^-) was generated from autoxidation of hematoxylin and was detected by an increasing absorbance at 560 nm wavelength in a UV-visible spectrophotometer (ELICO, S L164). The reaction mixture contained 0.1 M phosphate buffer (pH-7.4), 0.1mM EDTA, 50µM hematoxylin and 25µl of extract. The final volume of the reaction mixture was adjusted to 2.5ml by adding double distilled water[32]. The inhibition of autoxidation of hematoxylin in presence of extract over the control was calculated.

Hydroxyl ion Generation : Hydroxyl radical was generated from Fe^{2+}-ascorbate-EDTA-H_2O_2 system

(Fentons' reaction) which attacks the deoxy D-ribose and a series of reactions that eventually resulted in the formation of malonaldehyde (MDA). The reaction mixture contained 2.8 mM 2-deoxy D-ribose, 20mM of KH_2 PO_4 - KOH (pH-7.4), 100 mM $FeCl_3$, 104 µM EDTA, 1mM H_2O_2, 1mM ascorbic acid and 25 µl of turmeric extract. The reaction mixture was incubated at 37°C in humidified atmosphere containing 5% CO_2 in air for 1 hr. Then 2 ml of TBA-TCA reagent was added in each tube and boiled for 15 min. The color of the reaction mixture changes to a pink MDA-TBA chromogen which was finally measured at 532 nm in UV-spectrophotometer (ELICO, S L164). The level of hydroxyl radical generation was expressed as nM of MDA generated/hr[33].

Lipid Peroxidation : Lipid peroxidation of lymphocytes and tumor cells with the influence of turmeric extract was estimated separately according to Miller and Aust, 1989[34]. Lipid peroxidation was induced by copper - ascorbate system and estimated as thioburbituric acid reacting substances (TBARS). The thiobarbituric acid assay is the most frequently used method for determining the extent of membrane lipid peroxidation *in vitro*. Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535 nm.

The reaction mixture contained $1X10^6$ packed cells in 0.2M phosphate buffer pH (7.4), with 20mMTris-HCl, 2mM CuCl2, 10mM ascorbic acid and 25µl of ethanolic turmeric extract and were incubated for 1 hour at 37°C in humidified atmosphere containing 5% CO_2 in air. Lipid peroxidation was measured as malonaldehyde (MDA) equivalent using trichloroacetic acid (TCA), thiobarbituric acid (TBA) and HC1 (TBA-TCA reagent: 0.375% w/v TBA, 15% w/v TCA and 0.25 N HC1).

After incubation 2ml of TBA-TCA reagent was added and the mixture in each tube was shaken thoroughly. The tubes were then placed in a water bath for 15 min and then centrifuged for 10 min at 1000 g. Finally the supernatant from each tube was taken turn wise in a cuvette and the OD value was determined spectrophotometrically at 535 nm. Results of lipid peroxidation have been expressed as nmol MDA produced/hr/10^6 cells.

Nitric Oxide Synthase (NOS) activity : NOS activity was determined by measuring the conversion of oxyhemoglobin to methemoglobin according to Jia *et al.*[35]. L- arginine was found to be the precursor for the synthesis of NO by vascular cells. Cytosolic NAPDH dependent monoxygenase is responsible for the conversion of L-arginine to NO. L-arginine first undergoes monohydroxylation to N^G- hydroxyl-L-arginine which is then oxidized to L-citrulline and produces NO. This NO undergoes oxidation with oxyhemoglobin (HbO_2) and produces methemoglobin (met Hb). Thus the formation of metHb indicates the production of NO.

Briefly $1x10^6$ packed cells (lymphocytes or tumor cells) were incubated for 2 hr with 50 mM Tris-HCl buffer (pH 7.4), 10mM L-arginine, 64mM hemoglobin, with 25 µl of turmeric extract at 37°C in humidified atmosphere containing 5% CO_2 in air. After incubation,reaction mixture was centrifuged at 1000rpm for 5 min and the optical density of supernatant was measured in UV- spectrophotometer (ELICO, S L164) at 535nm. Results of NO production were expressed as pmol of NO produced/hr.

To confirm that the production of NO was actually due to the activation of NOS, a competitive inhibitor of nitric oxide synthase (NOS), 10µM N^G methyl- L-arginine acetate ester (NAME) was added in a separate set of experimental tubes.

Scanning Electron Microscopy (SEM) : Cells treated earlier with turmeric extract (25 µl) *in vitro* were fixed in Karnovsky fixative for 3 to 4 h at 4°C. A drop of cell suspension was taken on clean glass stubs (approximately 18x18 mm) and waited for 5-10 min to allow the cells to settle down which were then air dried. The cells were washed in cocodylate buffer twice for 10 min each dipping the whole stub containing the cells in buffer. The cells were then dehydrated with an ascending grade of acetone (30-50-70-80-90-95% twice for 10 min each) at room temperature and finally kept in dry acetone. After dehydration the cells were dried by critical point drying method, substituting dry acetone from the cells by carbon dioxide. After drying, cells were coated with gold in a fine coat ion sputter (J.C.F. 1100) by mounting the glass stub containing the cells on a brass stub with electro-conducting paints. Cells were then examined and photographed under Scanning electron microscope (Leo 435 VP) at AIIMS, New Delhi.

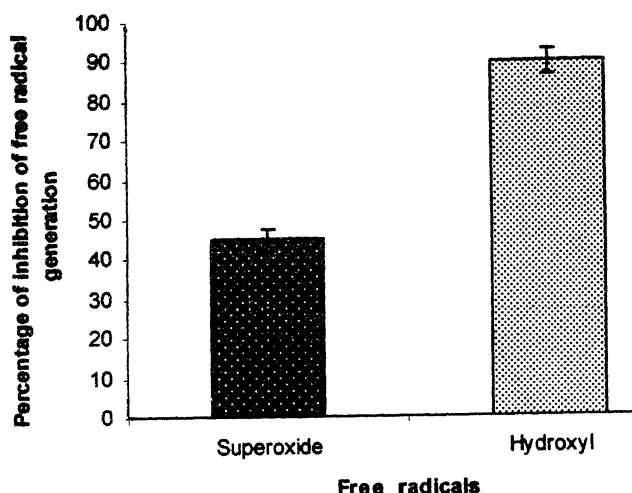



Fig. 1 - The percentage of inhibition of superoxide and hydroxyl ion generation by ETE treatment over the control (alcohol treated).

Fig. 2 - Copper ascorbate induced H_2O_2 generation in lymphocytes and tumor cells. Turmeric treatment shows lower production of MDA than the control.

Fig. 3 - L- arginine derived NO production in both lymphocytes as well as tumor cells. Activation of NOS by ETE treatment, as suggested by higher production of NO than the control.

Statistical analysis : Each experiment was performed in triplicate and an experiment was repeated thrice or more. This was applicable for all experiments. Results are expressed as Mean±SD of *n* observations. Statistical significance was analyzed using ANOVA software package.

Results and Discussion

Superoxide is the most abundantly produced free radical which dismutases into molecular oxygen and hydrogen peroxide in the presence of proton. This hydrogen peroxide then induces cellular damage in the presence of ferrous ions by a Fenton reaction, resulting in the formation of OH^- free radicals and further aggravates the peroxidation of lipid membrane.

Generation of superoxide from autoxidation of hematoxylin in the assay was inhibited by ethanolic turmeric extract (ETE) to 45% over the control (Fig. 1). ETE also inhibits hydroxyl ion generation by 89% over the control, suggesting its potent antioxidant activity (Fig:1). Furthermore, turmeric inhibits copper-ascorbate induced lipid peroxidation in lymphocytes and tumor cells as revealed by MDA production (Fig: 2). MDA production indicates the level of lipid peroxidation, i.e. H_2O_2 generation. In the presence of turmeric, inhibition of H_2O_2 generation in lymphocytes was maximal (43.33%) in reference to the control. The corresponding value of tumor cell is 11.64%. Thus, it gives more protection to the normal lymphocytes from lipid peroxidation and probably for this reason, better survivality of normal lymphocytes with turmeric treatment *in vitro* could be seen than the tumor cells[28-29].

Lipid peroxidation is actually the oxidative deterioration of polyunsaturated lipids. This leads to decrease in membrane fluidity, increase the leakiness of the membrane to substances such as Ca^{2+} and also inactive membrane- bound enzymes. Our results indicate that turmeric inhibits lipid peroxidation by scavenging and neutralizing free radicals, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). Thus, turmeric seems to function in maintenance of cell membrane integrity. The active component of turmeric

A

B

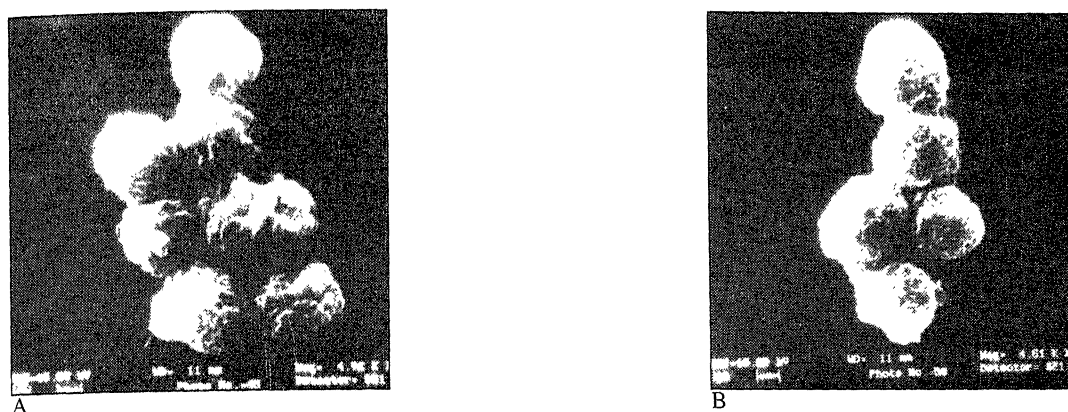



Fig. 4 - Scanning electron micrographs of murine lymphocytes from spleen after 16 hrs of *in vitro* treatment; A) Alcohol treated (control); B) Turmeric treated- cell maintained volume and shape, seemed to be cytoprotective.

A

B

Fig. 5 - Scanning electron micrographs of murine Ehlrich ascitic carcinoma cells after 16 hrs of *in vitro* treatment; A) Alcohol treated(control); B) Turmeric treated- cell surface irregularities indicating cytoplasmic blebs and probable entry of cells into apoptosis.

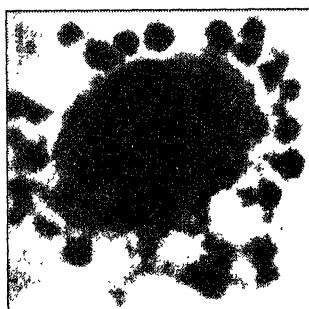



Fig. 6 - Tumor cells stained with Giemsa under light microscope, showing formation of multiple apoptotic bodies around the cell. x 2400.

is curcumin, its phenolic OH group and β- diketone group[36] have been suggested to contribute towards antioxidant property.

NO is pleiotropic molecule and mediates diverse functions by acting in most cells of the body through interaction with different molecular targets from superoxide anion to protein macromolecules, which can either be activated or inhibited through oxidation of thiols, hemes, Fe-S clusters, and other nonheme iron prosthetic groups of macromolecules[37-42]. This NO being designated as a messenger molecule of different biological functions[43-44] can also act as protector from cytotoxicity associated with oxyradical[45]. L-arginine derived NO production was observed in both lymphocytes as well as tumor cells with turmeric treatment. Normal lymphocytes produced 77.72 pmol of NO/mg dry wt. /hr with turmeric treatment, whereas lymphocytes collected from tumor bearing mice produced 194.09 pmol/mg dry wt/hr of NO. Tumor cells treated with ETE produced 189.09 pmol/mg dry wt./hr of NO. Control values (alcohol treated and untreated) showed very little generation of NO (Fig: 3). Addition of 10μM N^G methyl- L-arginine acetate ester (NAME) to the reaction mixture in all the cases completely inhibited NO production, thus suggesting that NO production was due to the NOS activity.

NO production in lymphocytes treated with turmeric seems to stimulate lymphocytes. Turmeric is stimulatory for murine lymphocytes by activating the cells towards blastogenesis, increasing ^{3}H- thymidine incorporation and was also found to drive the lymphocytes towards mitotic stage as observed by FACS[29]. Lymphocytes under scanning electron microscopy also showed healthy nature by maintaining its normal volume and shape with 16 hrs of *in vitro* ETE treatment, suggesting the cytoprotective nature of turmeric towards lymphocytes (Fig. 4A-B). Higher production of NO by lymphocytes taken from tumor bearing mice also suggests the possible involvement of NO mediated activation of cytotoxic response towards tumor cells.

L-arginine derived NO production is also critical for tumor cells[46-47]. Excessive NO results in limitation of angiogenesis and in some tumor cells increases apoptosis[48-49]. Furthermore, the arrest of tumor cells at S phase with turmeric treatment has also been observed under FACS[29]. NO generated with the treatment of turmeric, is distinctly inducing the tumor cells towards apoptosis as revealed in the light microscopic and scanning electron microscopic studies (Fig. 5A-B & 6)

The present study establishes the cytoprotective nature of turmeric for lymphocytes. This is possible due to inhibition of the lipid peroxidation and cell membrane protection by the turmeric. Simultaneously it increases the level of NO production which has been relevant for activating lymphocytes for mounting cytotoxic response towards tumor cells. On the other hand turmeric can directly affect the apoptotic process of tumor cells.

Acknowledgement

The authors acknowledge the help of Dr. K. Acharya, Department of Botany, Darjeeling Government College for developing the assays for biochemical estimation of free radicals. They are also thankful to the technical staff of Electron Microscopic unit of AIIMS, New Delhi and ICMR for the financial assistance.

References

1. Bandyopadhyay U, Das D, Banerjee RK. Reactive oxygen species: Oxidative damage and pathogenesis. *Current Science* 1999; 77 : 658-666.

2. Tiwari AK. Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidants therapy. *Current Science* 2001; **81** : 1179-1186.

3. Rahaman I, Swarska E, Henry M, Stolk J, MacNeed W. Is there any relationship between plasma antioxidant capacity and lung function in smokers and in patients with chronic obstructive pulmonary disease? *Thorax* 2000; **256**: 1-11.

4. MacNee W, Rahman I. Oxidants and oxidants as therapeutic targets in chronic obstructive pulmonary disease. *Am J Respire Crit Care Med* 1999; **160** : 558-565.

5. Rahman I, MacNee W. Lung glutathione and oxidative stress: implication in cigarette smoke-induced airway disease. *Am J Physiol, Lung Cell Mol Physiol* 1999; **277**: L1067-L1088.

6. MacNee W. Oxidative stress and lung inflammation in airway disease. *Eur J Pharmacol* 2001; **429** : 195-207.

7. Klaunig JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicolc* 2004; **44** : 239-67.

8. Simonian NA, Coyle JT. Oxidative stress in neurodegenerative diseases. *Annu Rev Pharmacol Toxicol* 1996; **36** : 83-106.

9. Chandel NS *et al.* Redox regulation of during hypoxia. *Oncogene* 2000; **19** : 3840-3848.

10. Vafa O *et al.* c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p^{53} function; a mechanism for oncogene induced genetic instability. *Mol Cell* 2001; **9** : 1031-1044.

11. Haddad JJ. Redox and oxidant-mediated regulation of apoptosis signaling pathways: immuno-pharmaco-redox conception of oxidative siege versus cell death commitment. *Immunopharmacol* 2004; **4** : 475-493.

12. Haddad JJ. The involvement of L-γ-glutaniyl-L-cysteinyl-glycine (glutathione GSH) in the mechanism of redox signaling mediated $MAPK^{p38}$ dependent regulation of pro-inflammatory cytokine production. *Biochem Pharmacol* 2002; **63** : 305-20.

13. Haddad JJ. Redox and oxygen sensitive transcription factors in the regulation of oxidant-mediated lung injury.I. Role for nuclear factor -κB. *Crit Cares* 2002; **6:** 481-490.

14. Haddad J, Olver RE, Land SC. Antioxidant pro-oxidant equilibrium regulates HIF-1α and NF-κB redox sensitivity: evidence for inhibition by glutathione oxidation in alveolar epithelial cells. *J Biol Chem* 2000; **275** : 21130-21139.

15. Thannekal VJ, Fanburg BL. Reactive oxygen species in cell signallin g. *Am J Physiol Lung Cell Mol Physiol* 2002; **279** : L1005-L1028.

16. Simon HU, Haj-Yehia A, Levi-Schaffewr F. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis* 2000; **5** : 415-418.

17. Naik SR. Antioxidants and their role in biological functions: an overview. *Indian Drugs* 2003; **40** : 501-516.

18. Kunchandy E, Rao MNA. Effect of curcumin on hydroxyl radical generation through Fenton reaction. *Int J Pharm* 1989; **57** : 173-176.

19. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. *Int J Pharm* 1990; **58** : 23-240.

20. Rajakumar DV, Rao MNA. Antioxidant properties of dehydrozingerone and curcumin in rat brain homogenates. *Mol and Cell Bioch* 1994; **140** : 73-79.

21. Toda S, Ohnishi M, Kimura M, Nakashima K. Action of curcuminoids on the haemolysis and lipid peroxidation of mouse erythrocytes induced by hydrogen peroxide. *J. Ethanopharmacol* 1998; **23** : 105-108.

22. Reddy AC, Lokesh BR. Effect of dietary turmeric *(Curcuma longa)* on iron-induced lipid peroxidation in the rat liver. *Chem Toxic* 1994; **32** : 279-283.

23. Cohly HHP, Taylor A, Angel MF, Salahudeen AK. Effect of turmeric, turmerin and curcumin on H_2O_2- induced renal epithelial (LLC-PK_1) cell injury. *Free Rad Biol Med* 1998; **24** : 49-54.

24. Brouct I, Ohshima H. Curcumin, an anti-tumor promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. *Bioch and Bioc Res Comm* 1995; **206** : 533-540.

25. Mazumdar A, Raghavan K, Weinstein J, Kohn KW, Pommier Y. Inhibition of human immunodeficiency virus type-I integrase by curcumin. *Biochem Pharmacol* 1995 **49** : 1165-1170.

26. Huang MT, Lou YR, Ma W, Newmark HL, Reuhl KR, Conney AH. Inhibitory effects of dietary curcumin on forestomach, duodenal and colon carcinogenesis in mice. *Cancer Res* 1994; **54** : 5841-5847.

27. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer Lett* 1995; **94** : 79-83.

28. Chakravarty A K, Yasmin H. Alcoholic Turmeric Extract Simultaneously Activating Murine Lymphocytes and Inducing Aopoptosis of Ehlrich's Carcinoma Cells. *International Immunopharmacology,* 2005; **5** : 1574-1581.

29. Chakravarty A K, Yasmin H. Two way efficacy of alcoholic turmeric extract : Stimulatory for Murine Lymphocytes and inhibitory for Ascitic Fibrosarcoma, *Pharmaceutical Biology* 2003; **42** : 217-224.

30. Lu YP, Chang RL, Lou YR, Huang MT, Newmark HL, Reuhl KR, Conney AH. Effect of curcumin on 12-*O*-tetradecanoylphorbol-13-acetate-and ultraviolet B light induced expression of c-Jun and c-Fos in JB6 cells in mouse epidermis. *Carcinogenesis* 1994; **15** : 2363-2370.

31. Khar A, Ali AM, Pardhasaradhi BV, Begum Z, Anjum R. Antitumour activity of curcumin is mediated through the induction of apoptosis in AK-5 tumour cells. *FEBS Lett* 1999; **445** : 165-168.

32. Martin JP, Daiby M, Sugrman E. Negative and positive assays of superoxide dismutase based on hematoxilin autoxidation. *Arch Bioch Bioph* 1987; **208** : 329-336.

33. Hallwell B, Guttendge JML, Aruma OI. The deoxynbose method, a simple "test tube assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem* 1987; **65** : 215-219.

34. Miller DM, Aust SD. Studies of ascorbate- dependent, iron-catalyzed lipid peroxidation. *Arch Biochem. Biophysics* 1989; **271** : 113-119.

35. Jia L, Bonaventua C, Bonaventura J, Stamler SJ. S-nitrosohemoglobulin: a dynamic activity of blood involved in vascular control. *Nature* 1996; **380** : 221-226.

36. Tonnensen HH, Arrieta AF, Lerner D. Studies on curcumin and curcuminoids, XXIV. Characterization of the spectroscopic properties of the naturally occurring curcuminoids and selected derivatives. *Pharmazie* 1995; **12** : 689-693.

37. Fleming INO: the primary EDRF. *J Mol Cell Cardiol* 1999; **31** : 5-14.

38. Ignarro L J. Physiology of nitric oxide. *Kidney Int Supply* 1996; **55** : S2-S5.

39. Moncado S, Higgs EA. Molecular mechanisms and therapeutic strategies related to nitric oxide. *FASEB,* 1995; **9** : 1319-1330.

40. Furchgott RF, Jothianandan D. Endothelium-dependent and independent vasodilation involving cyclic GMP; relaxation induced by nitric oxide, carbon monoxide and light. *Blood Vessels* 1991; **28** : 52-61.

41. Nathan C, Nitric oxide as secretory product of mammalian cells. *FASEB* 1992; **6** : 3051-3065.

42. Moncada S. Nitric oxide: discovery and impact on clinical medicine. *J R Soc Med* 1999; **92** : 164-169.

43. Bredt DS, Snyder SH. Nitric oxide: a physiologic messenger molecule. *Annu Rev Biochem* 1994; **63** : 175-195.

44. Gladwin MT, Crawford JH, Patel RP. The biochemistry of nitric oxide, nitrite, and hemoglobin; role in blood flow regulation. *Free Rad Biol Med* 2004; **6** : 707-717.

45. Ignarro LJ. Biological actions and properties of endothelium- derived nitric oxide formed and released from artery and vein. *Circ Res* 1989; **65** : 1-21.

46. Hibbs JB, Taintor RR; Vavrin Z. Macrophage cyotoxicity: role for L-arginine deiminase activity and amino nitrogen to nitric. *Science* 1987; **235** : 473-476.

47. Xie K, Fidler IJ. Therapy of cancer metastasis by activation of the inducible nitric oxidesynthase. *Cancer Metastasis Rev* 1998; **17** : 55-75.

48. Hung S, Xie K. Contribution of nitric oxide mediated apoptosis to cancer metastasis inefficiency. *Free Rad Biol Med* 2003; **34** : 969-986.

49. Hofseth LJ *et al.* Nitric oxide in cancer and chemoprevention. *Free Rad Biol Med* 2003; **34** : 955-965.

Reproductive biology of *Parkinsonia aculeata* L. (Caesalpinaceae)

SEEMA CHAUHAN, SHASHI BALA SHARMA and S.V.S. CHAUHAN*

Department of Botany, School of Life Sciences, Dr.B.R. Ambedkar University, Agra-282002, India.

**e-mail: svs250@rediffmail.com*

Received August 3, 2006, Revised April 12, 2007, Accepted September 24, 2007

Abstract

Parkinsonia aculeata L. (Caesalpinaceae), a small xerophytic tree species, flowers twice a year, during March-April and August-September with profuse flowering in the former period. The flowers, arranged in lax axillary racemes are yellow, hermaphrodite, hypogynous, zygomorphic and complete. They open daily during 5.00-6.30 am followed by anther dehiscence at 6.00-7.30 am. There are 10 stamens (4 long, 4 medium and 2 small staminodes) and each flower produces 65040 ±1201 pollen grains. Temperature and relative humidity have direct effect on pollen viability, which is low during March-April and high during August-September. The flowers offer both pollen and nectar for the visitors, which include honeybees, butterflies, wasps and ants. Fruiting in this species is through autogamy, geitonogamy and xenogamy, however, it has predominantly facultative xenogamous breeding system. This system helps it to be adaptive for colonization.

Key words: *Parkinsonia aculeata, flora biology, breeding systems, pollination.*

सारांश

पारकिन्सोनिया सिसिलपिनेसी कुल का एक छोटा मरूद्भिदी वृक्ष है। यह वर्ष में दो बार (मार्च–अप्रैल तथा अगस्त–सितम्बर) में पुष्पधारण करता है, जिसमें अधिक पुष्प मार्च–अप्रैल में बनते है। जायांगधर पीले पूर्ण पुष्प अक्षतः असीमाक्ष पुष्पक्रम में लगे रहते हैं जो प्रतिदिन प्रातः 5–6 बजे के बीच खिलते है। इसके उपरान्त परागकोष का स्फुटन प्रातः 6–7.30 बजे के मध्य होता है। पुंकेसर की संख्या 10, जिनमें 4 बड़े, 4 मध्यम व 2 छोटे बध्य पुंकेसर होते है। प्रत्येक पुष्प में 65040 ± 1201 परागकण बनते हैं। तापक्रम व आर्द्रता का सीधा असर परागकण की जीवनक्षमता पर पड़ता है। मार्च–अप्रैल में कम तथा अगस्त–सितम्बर में अधिक होती है। पुष्प, आंगुन्तकों के लिये जिसमें मधुमक्खी, तितली, बर्र तथा चीटियाँ सम्मिलित हैं, परागकण तथा मंकरन्द प्रदान करते है। इस वृक्ष में फल स्वयुग्मन, सपादपी उतर–परागण तथा परनिषेचन द्वारा बनते है। परन्तु यह वृक्ष मुख्य रूप से विकल्पी परनिषेचन प्रजनन तंत्र प्रदर्शित करता है। यह तंत्र इस वृक्ष के उपनिवेशन के लिये अनुकूल होता है।

सांकेतिक शब्द : *पार्किन्सोनिया एक्यूलियारा,* पादप जीव विज्ञान, प्रजनन व्यवस्था, परागनिषेचन।

Introduction

Parkinsonia aculeata L. (Caesalpinaceae), a native of Panama, is a small spinous xerophytic tree, commonly known as 'Vilayati Kikar'. It is an important plant of semi-arid zone and is quite common in Agra and Bharatpur regions, which is popularly known as Brij Mandal. The wood is commonly used as fuel but also yields good charcoal and the leaves provide fodder. Due to rapid urbanization and industrialization, this species is being cut ruthlessly and no measures for its conservation have been undertaken. Owing to these constraints it is disappearing from the local habitat. Reproductive biology plays an important role in the conservation of biodiversity, particularly those of endangered species, endemic species of medicinal importance and plants of other economic importance. Angiosperm tree species tend to be out-crossing and have lower reproductive efficiency[1]. Therefore, in order to conserve this important tree of Brij Mandal a detailed study on its reproductive biology has been undertaken.

Material and Methods

Present investigation was carried out on fifty marked trees of *P. aculeata L.*, growing at five different parts of Agra district during 2004-2005. Observations were made on the timings of leaf fall, leaf renewal, flowering and fruiting events. Fifty flowers collected from different trees were used to record the floral morphometries. Morphology of pistil was also studied under the scanning electron microscope. Fresh pistils were fixed in 3% glutaraldehyde in 0.1M phosphate buffer, dehydrated through aqueous acetone series, dried with CO_2 in a HCP-2 Hitachi Critical point dryer using liquid CO_2 at 1000 Ibs per inch. The samples were mounted on

stubs, coated with gold (20 mm) and stored in a dessicator. They were observed and photographed using Philips EM 501 SEM at All India Institute of Medical Sciences, New Delhi.

The time of daily anthesis and anther dehiscence was recorded. Number of pollen grains/anther/flower was determined from 150-flowers/marked trees following the procedure after Cruden[2]. Pollen size was measured under a light microscope after McKone and Webb[3]. Stigma receptivity was determined by placing fresh pollen on the stigma of flowers developed from the emasculated and bagged floral buds. These were periodically observed for pollen germination under a microscope. The number of pollen grains divided by the number of ovules per flower yielded the pollen-ovule ratio according to procedure after Cruden[2]. The pollen viability was assessed by both *in vitro* and *in vivo* pollen germination studies. *In vitro* pollen germination was studied by hanging drop method after Brewbacker and Kwack[4] and *in vivo* germination by aniline blue fluorescence microscopic method as described by Shivanna and Rangaswammy[5]. Breeding behaviour by autogamy (bagged and hand pollinated), geitonogamy and xenogamy was tested by controlled pollination studies. In order to observe the rate of natural fruit-set, one hundred inflorescences on different trees were tagged and were followed until fruit development. The flower visitors were the insects. Their activities were observed using binocular. The insects were collected and identified. The daily foraging frequency and probing behaviour of different foragers were recorded. Pollination efficiency of different insects was checked by observing the pollen load on different body parts under light microscope according to procedure given by Kearns and Inouye[6]. In order to determine the true pollinator, the stigmatic surface was observed under dissecting microscope to determine the pollen load before and after the single visit of the insect.

Results and Discussion

Flowering in *Parkinsonia aculeata* L. occurs in two phases in a year. During the first phase, at temperature ranging between 35°C-43°C with 20%-40% relative humidity, flowering starts in the second week of March and continues till the last week of April with maximum flowering during the first week of April (Fig.1A). On the other hand, during the second phase, at temperature between 23°C-31°C and 60%-80% relative humidity, flowering again starts in the second week of August

Table 1 - Floral characters of *Parkinsonia aculeata.*

Floral characters	Observations
Flowering period	March-April, August-September
Inflorescence	Axillary raceme
No. of flowers/ Inflorescence	20±2.5 (Range 16-24)
Flower	Hermaphrodite, zygomorphic, hypogynous
No. of stamens	10(4L,4M&2S)
Calyx	Gamosepalous with five sepals
Corolla	Polypetalous with five petals
Time of flower opening	5.00-6.30 am
Time of anther dehiscence	6.00-7.30 am
Mode of anther dehiscence	Apical pore
Pollen grains/ flower	65040±1201 (62010-68032)
Pollen grains/long stamen	10210±3510(8105-1211)
Pollen grains/medium stamen	6050±1125 (4015-8090)
Stigma	Unifid, wet & papillate
Time of stigma receptivity	8.00-12.00 am
Ovary	Monocarpellary, superior, unilocular
No. of ovules/ovary	10±2.5 (Range 8-12)
Ovule type	Campy lotropous

± - Standard deviation, L- long stamen, M-medium sized stamen, S-staminode

and continues till the last week of September with maximum flowering during the first week of September (Fig.1B). However, the floral density (both number of inflorescence/plant as well as number of flowers/ inflorescence) is significantly higher during the first phase. The flowers are arranged in lax axillary racemes and each raceme consists of 20±2.5 flowers (Range 16-24) (Fig. 1C). Flowers are yellow, pedicellate, bracteate, hermaphrodite/ hypogynous, zygomorphic and complete (Table 1). The calyx is gamosepalous with five sepals; odd sepal anterior in position. The corolla is polypetalous with five petals; odd posterior petal smallest and innermost. This petal bears dark red spots. There are ten stamens, of which four are long (0.8±0.3cm), four medium sized (0.6±0.2cm) and two small (0.4±0.1cm) which are reduced to petaloid staminodes. Anthers of

long stamens measure 0.5±0.15cm, while those of the medium sized stamens are 0.3+0.1cm long. Staminodes fail to show any differentiation into anthers and filaments. Pistil is single and is red in colour. It is differentiated into a unifid, papillate and wet stigma, which projects beyond the level of the anthers, a short style and monocarpellary, superior and unilocular ovary (Fig. 2A-C, Table 1). There are 10±2.5 (Range 8-12) campylotropous ovules lying on marginal placentae. Numerous trichomes are observed on the ovarian surface (Fig. 2C). Ovarian trichomes have also been observed in other members of the family Caesalpiniaceae e.g. *Cassia occidentalis* L.[7] and *Cassia tora* L.[8].

The flowers open daily during 5.00-6.30 am and the anthers dehisce through apical pores around 6.00-7.30 am (Table 1). The pollen grains are spherical and tricolpate with reticulate exine and are 30µm in diameter. The number of pollen grains/flower is 65040±1201 (Range 62010-68032), in which long stamen contributes 10210±3510 (Range 8105-1211) pollen grains/anther, whereas, medium sized stamen contributes 6050±1125 (Range 4015-8090) pollen grains/anther. Staminodes are sterile and devoid of pollen grains. Floral biology of *Cassia didymobotrya* L. and C. *auriculata* L. of family Caesalpiniaceae has been studied by Dulberger[9]. According to him, there are three types of anthers that differ in size and pollen production and dehisce through apical pores.

The pollen-ovule ratio is 6504:1. The stigma becomes receptive after anther dehiscence around 8.00 am and remains so until 12.00 noon of the same day. *In vitro* pollen germination studies indicate that the pollen grains remain viable for 6h after anther dehiscence. Percentage of pollen viability is higher during August-September as compared to March-April. Among the anthers, long anther exhibits higher pollen viability than the medium sized anther. At the time of anther dehiscence, during the first phase long and medium sized anthers exhibit 98% and 95% pollen viability, whereas during the second phase pollen viability in long and

Table 2 - Results of fruit-set in *P. aculeata*.

Treatments	No. of flowers pollinated	No. of flowers forming fruits	Fruit-set (%)	No. of fruits dropped permanently	Fruit drop (%)
1. Autogamy					
i. Unmanipulated	50	0	0	0	0
ii.. Manipulated	50	18	36	14	78
2. Geitonogamy	50	40	80	18	45
3. Xenogamy	50	45	90	0	0

Table 3 - Pollen pick-up by insects on *P. aculeata* as revealed by observing pollen on different body parts.

Visitor species	Sample size	Pollen Grains	
		Range	Mean
Apis dorsata	10	445-515	477±36.5
A. indica	10	350-390	366±32.3
Pieris brassicae	10	320-340	331±27.6
Danaus plexippus	10	250-290	270±21.0
Polistes hebraeus	10	200-240	216±17.6
Vespa sp.	10	140-170	157±9.5
Camponotus compressus	10	80-130	106±4.5

± : Standard deviation

medium sized anthers is 80% and 77% respectively. However, there is a substantial reduction in pollen viability afterwards-2h (75% and 72%), 4h (45% and 42%) and 6h (11% and 8%) in long and medium sized anthers respectively during the second phase. On the other hand, during the first phase, pollen viability of long and medium sized anthers after 2, 4 and 6h is 61% and 59%, 37% and 34% and 5% and 2% respectively.

In vivo pollen germination studies on the stigmatic surface during the second phase show 80% and 77% pollen germination after 2h, 49% and 46% after 4h and 14% and 12% after 6h after hand pollinating the stigma with the pollen of long and medium sized anthers. On the other hand, during the first phase, there is 65% and 63% pollen germination after 2h, 42% and 39% after 4h and only 7% and 4% after 6h on the stigmatic surface by the pollen of long and medium sized anthers respectively.

Fruiting starts in the first week of April and the fruits mature within 5-6 weeks. Fruit is a pod. It is green when young and turns gradually to light brown upon maturity (Fig. ID). Mature fruits start to disperse their seeds during the last week of June with the help of squirrels and birds. Fruiting again starts in the first week of September and mature fruits starts to dehisce during the first week of December. A limited number of mature fruits remain attached on the tree till the next flowering season. The hand pollination tests for breeding systems indicate 36% fruit-set through autogamy, 80% fruit-set through geitonogamy and 90% fruit-set through xenogamy. Most of the autogamous and geitonogamous fruits dropped-off prematurely, while all xenogamous fruits remain attached till maturity (Table 2). Natural fruit-set is 76% during the second phase. A sample of 100 inflorescences consisting of 2024 flowers with 20240 ovules selected at random on selected trees at flower stages was used for estimating fruit and seed-set rate. Among these, 1540 flowers with 15400 ovules set fruits with 8310 seeds. Seed-set is 53%. In the remaining flowers, some pollinated flowers initially develop fruits but later aborted. On the other hand, during the first phase, fruit-set is reduced to 64% with 41% seed-set. Over production of flowers is a quite common phenomenon in angiosperms[10, 11]. A study on the cost of advertisement in plants has shown that in *Cassia fistula* L., there is a significant positive association between the attractiveness of the trees and damage by insects[12]. The attractiveness of the trees is also positively correlated with the fruit set percentage[12].

The flowers are visited by honeybees *(Apis dorsata* and *Apis indica* Fabr.), wasps *(Polistes hebraeus* Fabr. and *Vespa* sp.) and ants *(Camponotus compressus* F. Muell.). They forage daily during daytime hours from 5.00 am-1.00 pm with peak frequency during 8.00-11.00 am. They forage for both pollen and nectar and of total visits, honey bees made 60%, wasps 22% and ants 18%. Observation of different body parts of the insects under microscope indicate that *Apis dorsata* Fabr. have higher amount of pollen on their body than the other insect specimens (Table 3). All the visitors are pollinators because considerable amount of pollen grains were observed on the stigmatic surface even after the single visit of a particular insect. After single visit of *Apis dorsata* Fabr., 234 pollen grains were recorded on the stigmatic surface. On the other hand, after single visit of *Apis indica* Fabr., *Polistes hebraeus* Fabr., *Vespa* sp. and *Camponotus compressus* F. Muell, the stigma was loaded with 155, 42, 26 and 15 pollen grains respectively. Since the pollen load on the stigmatic surface after the visit of *Apis dorsata* Fabr. is higher as compared to other insects, it is proved be the main pollinator. This is because of its frequent intra- and inter-tree movement.

Honeybees probe the flower in an upright position. While, alighting on the flower, they first contact the stigma and then the stamens with their underside. In effect, the bees pick up pollen on their pollen baskets on tarsi of legs. This pollen gets transferred to other conspecific stigmas in their subsequent visits. The bees collect the nectar from the flower base. Wasps and ants also use the same posture that the bees employ and are also the pollen carriers. Ants are found moving within and among the flowers of the same individual by staying on the plant all the time, effecting autogamy and geitonogamy. On the other hand, wasps facilitate both self- and cross-pollinations by their intra-and inter-plant movements. Observations of different body parts of the insects under microscope indicate that *Apis dorsata* Fabr. have higher amount of pollen on their body than the other insect specimens (Table 3). It is dominant in number and is the main pollinator because of its frequent intra-and inter-tree movements.

In P. *aculeata* there are ten stamens of unequal sizes. Heterostameny in some *Cassia* species has also been observed[13]. The heteroantherous flowers have unusually large and showy anthers that contain copious pollen for dispersal, and fodder stamens, which act as food for the pollinators[14]. Bees are also attracted by the yellow colour of the flower. The floral colour is an important

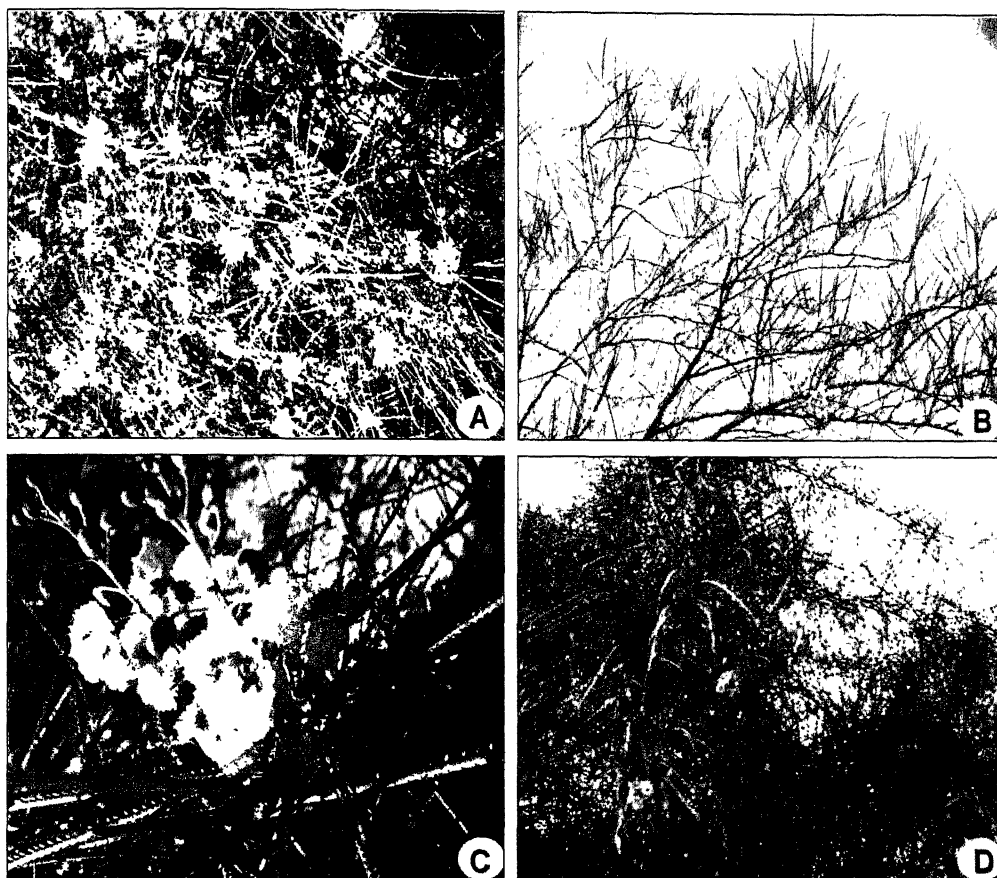



Fig.1 A-D - *Parkinsonia aculeata* L showing floral density and fruits.

A. Floral density during March-April. **B.** Floral density during August-September. **C.** Flowers arranged in lax axillary racemes. **D.** Fruits along with a few flowers.

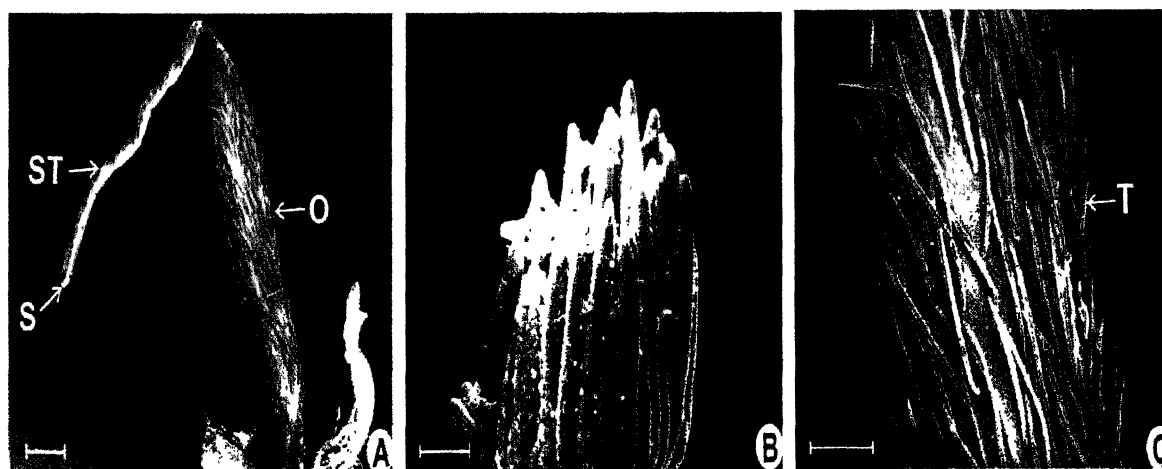



Fig 2 A-C. - Scanning electron microphotographs of pistil.

A. Pistil showing stigma (S), style (ST) and ovary (O), Bar 300mm. **B.** Magnified view of stigmatic surface showing elongated papillae. Bar 20mm **C.** Magnified view of ovarian trichomes (T). Bar 100mm

attractant and bees in general have been reported to be associated with the flower species possessing blue or yellow colour[15]. *Senna acclinis* F. Muell. (Caesalpiniodeae) is known to be pollinated by a taxonomically diverse assemblage of generalist native bees, namely, *Hylaeus turgicollaris* Michener., *Amphylaeus* sp, *Lasioglossum polygoni* Cockvell., *Lasioglossum victoriellum* Cockvell., *Lasioglossum (Parasphecodes)* sp. and *Exoneura* sp[16].

P. aculeata fruits through autogamy, geitonogamy and xenogamy, indicating that it has facultative xenogamous breeding system. Autogamy fails to take place without the pollen mediation by the flower visitors. This is because the automatic contact between the anthers and the stigma within the same flower does not take place as the stigma is placed well beyond the anthers. However, hand pollination of emasculated and subsequently bagged floral buds of its own pollen resulted in the formation of fruits. Therefore, both self- and cross-pollinations depend on pollen mediation performed by the flower visitors. Although, this species is both self- and cross-pollinated, it sheds most of the autogamous and geitonogamous fruits, while retaining all xenogamous fruits to maturity. This suggests that *P. aculeata* is predominantly cross-pollinated and leaves the possibilities for self-pollination. This species might be selectively eliminating the growing self-pollinated off springs in order to allocate resources for the xenogamous fruits. It has been predicted that pollen-ovule ratio is the indicator of breeding system, and further estimated the pollen-ovule ratio for each breeding system[2]. The pollen-ovule ratio found in *P. aculeata* is more than that predicted for facultative xenogamy. The high pollen-ovule ratio appears to be imperative for the success of facultative xenogamy, especially for xenogamy. A similar phenomenon has also been reported in *Pterocarpus santalinus* L., an endemic and endangered tree species[17].

The natural fruit-set rate indicates that the plant does not suffer seriously from under-pollination. However, the plant with predominant xenogamy requires mostly xenogamous pollen for more fruit-set, after selective elimination of growing fruit. Therefore, pollen transfer between conspecifics has a great bearing on the net percentage of natural fruit-set.

References

1. Sedgley M, Griffin AR. Sexual Reproduction of Tree Crops. London : Academic Press, 1989.
2. Cruden RW. Pollen ovule ratio: a conservative indicator of breeding system in flowering plants. *Evolution* 1977; **31** : 32-46.
3. McKone MJ, Webb CJ. A difference in pollen size between the male and harmaphrodite flower of two species of Apiaceae. *Australian J Botany* 1988; **36** : 331-337.
4. Brewbaker JL, Kwack BH. The essential role of calcium ion in pollen germination and pollen tube growth. *American J Botany* 1963; **50** : 859-865.
5. Shivanna KR, Rangaswamy NS. Pollen Biology: A Laboratory Manual, New Delhi; Narosa Publishing House, 1992.
6. Kearns CA, Inouye DW. Techniques for Pollination Biologist, Niwot, Colorado; University Press of Colorado, 1993.
7. Sharangpani PR, Shirke DR. Scanning electron microscopic studies on ovarian nectarines of *Cassia occidentalis L. Phytomorphology* 1996; **46** : 277-281.
8. Sharma SB, Rana A, Chauhan SVS. Reproductive biology of *Cassia tora* L. *Annals Forestry,* 2005; **13** : 63-66.
9. Dulberger R. The floral biology of *Cassia didymobotrya* and *Cassia auriculata. American J Botany* 1981; **68** : 1350-1360.
10. Stephenson AG. When does out crossing occur in a mass-flowering plants? *Evolution* 1982; **36** : 762-767.
11. Sutherland S. Pattern of fruit-set: what controls fruit-flower ratios in plants? *Evolution* 1986; **40** : 117-128.
12. Vasudeva R, Ganeshaiah KN, Shankar UR. Coast of advertisement in plants: Floral predation in *Derris indica* and *Cassia fistula.* In: Veeresh GK, Shanker UR, Ganeshaiah KN, editors. Pollen in Tropics. Bangalore : University of Agricultural Sciences, 1993; 6-8.
13. Chauhan SVS, Anuradha, Singh J. Stamen dimorphism in three *Cassia* species. *Phytomorphology* 2003; **53** : 173-178.
14. Buchmann SL. Buzz pollination in Angiosperms. In: Jones CE, Little RJ. editors. New York : Scientific and Academic Edition, 1983; 73-113.
15. Kevan PG. Handbook of Experimental Pollination Biology. In : Jones CE, Little R J. editors. New York: Scientific and Academic Editions, 1983; 3-30.
16. Williams G. Bee pollination in the threatened Australian shrub *Senna acclinis* (Caesalpinidae). *Cunninghamia* 1998; **5** : 767-772.
17. Rao SP, Solomon Raju AJ. Pollination ecology of the red sanders *Pterocarpus santalinus* (Fabaceae), an endemic and endangered tree species. *Curr Sci* 2002; **83** : 1144-1148.

Proc. Nat. Acad. Sci. India, Sect. B, Vol. 78 Pt. I, 2008

Occurrence of AM fungi at varying stages of growth of rice plants

ARCHANA DUBEY, MAHENDRA KUMAR MISHRA, PRADEEP KUMAR SINGH and DEEPAK VYAS*

Laboratory of Microbial Technology and Plant Pathology, Department of Botany, Dr. H.S. Gour University, Sagar-470003, India

**e-mail: dvyas64@yahoo.co.in*

Received July 25, 2006, Revised December 7, 2006, Accepted March 26, 2007

Abstract

In the present study the occurrence of AM fungi on rice plants at varying stages of growth was observed. At the seedling stage (40 DAS) 15 AMF species, 60% root colonization and arbuscules in good amount were observed. But at maturation stage (80 DAS) when plants were submerged in water, not only species of AMF reduced but also per cent of root colonization decreased and no arbuscules were seen. At harvesting stage (120 DAS), AM fungal species and percent root colonization increased. Vesicles were formed at this stage. 22 AMF species were recorded, and 8 AMF species were found common at all stages. Species of *Gigaspora* were found at seedling stage and 8 AMF were recorded only at harvesting stage. From the result it can be deduced that *Glomus* species was dominant in rhizosphere of rice plants followed by *Acualospora, Gigaspora* and *Scutellospora* which were poorly distributed.

Keywords : AM Fungi, colonization, spore population, arbuscules, vesicles.

सारांश

चावल के पौधों में वृद्धि की विभिन्न अवस्थाओं में अरबस्कुलर माइक्रोराइज़ा कवक (AM Fungi) का अध्ययन किया गया है। सीडलिंग अवस्था (40 दिनी पौधे) के दौरान 15 AMF स्पीसीज़ एवं 60% जड़ों में संक्रमण देखा गया। आरबस्क्यूल की मात्रा सबसे ज्यादा इसी अवस्था में देखी गयी। परिपक्वता की अवस्था (80 दिनी पौधे) में जब पौधे जल में डूबे हुये थे, उस समय न केवल AMF स्पीसीज की संख्या कम देखी गयी वरन् जड़ों में संक्रमणता भी कम देखी गयी और अरबस्क्यूल नहीं देखे गयें। 120 दिनी पौधों में फसल कटने के वक्त पर देखा गया कि जड़ों में संक्रमणता भी बढ़ी हुयी है और वेसाइकिल भी उपस्थित है। इनमें कुल 22 AMF रिकार्ड की गयी है। इस अध्ययन के दौरान 8 AMF स्पीसीज़ ऐसी थी जिनमें तीनों अवस्थायें पाई गयीं। *गीगास्फेरा* केवल स्पीडलिंग अवस्था में देखने में आयी। 8 AMF स्पीसीज़ केवल फसल कटने की अवस्था में देखने को मिली। उपलब्ध परिणामों से यह स्पष्ट होता है कि *ग्लोमस,* चावल के राइज़ोस्फीयर में सबसे ज्यादा संख्या मे हैं, उसके बाद *आकायलोस्पोरा* तथा *गीगास्पोरा* और *स्क्यूटिलोस्पोरा* सबसे कम संख्या में मौजूद है।

सांकेतिक शब्द : ए0एम0 कवक, उपनिवेशी प्रक्रिया, स्पोर–संख्या, अरबस्कूल, वेसिकिल्स।

Introduction

Rice (paddy) is an important food crop of India and it occupies an area of about 41 millions hectares. Rice is grown in humid areas of Assam, Manipur, West Bengal, Orissa, Bihar, Chhatisgarh, Eastern UP, South India, and Madhya Pradesh. Though Sagar region is predominantly known for wheat and soybean crops, yet there are a few blocks where farmers have started cultivation of rice.

Wetland rice systems in Asia are making a major contribution to global rice supply[12]. These systems are excellent examples of sustainable soil fertility maintenance[13]. A unique feature of soils that remain flooded for prolonged periods is the maintenance of soil fertility and productivity.

Arbuscular mycorrhizal (AM) fungi colonize in the roots of the majority of land plants, facilitate plant mineral nutrient uptake[4] and suppress plant diseases[5]. They are one of the oldest terrestrial organisms. Their fossil record dates back to the Ordovician, 460 million years ago[6,7]. Fossil evidence for sexual reproduction in ancestral AM fungi are lacking, but they represent one of the oldest groups of clonally reproducing eukaryotic organism on earth[8]. They account for up to 50% of the total soil microbial biomass[9] and contribute to the maintenance of soil aggregate structure[10]. Because of these characteristics, AM fungi are perceived as one of the most important components of a paradigm shift from conventional to sustainable land management practices[11].

Table 1 - Occurrence of AM fungi in Paddy fields of Sagar (in India)

AMF association						
Crop interval of collection (DAS)	Spore Population 100gm^{-1} soil	% Colonization	Intensity of arbuscules cm^{-1} root length	Intensity of vesicles cm^{-1} root length	Total No. of AM spp.	Name of AM species
40	813	60	+++	-	15	ABRT, ASCB, CGRG, CHTG, GABD, GRSA, LAGR, LCLD, LCLR, LFSC, LHOI, LHTS, LINR, LMSS, LOCT
80	320	29	-	+	9	ABRT, ASPN, LAGR, LCLR, LFSC, LHOI, LHTS, LMSS, LOCT
120	996	75	-	+++	22	ABRT, ADLC, ASCB, ASPN, CGRG, CHTG, LABS, LAGR, LCLD, LCLR, LDMR, LDST LFSC, LHOI, LHTS, LINR, LMSS, LMST, LOCT, LPST, LRBF, LSNS

ABRT = *Acaulospora beriticutata* (Rothwell and Trappe)
ASCB = *A. scrobiculata* (Trappe)
ASPN = *A. spinosa* (Walker and Trappe)
ADLC = *A. delicata* (Walker, Pfeiffer and Bloss)
LAGR = *Glomus aggregatum* (Schenck and Smith emend Koske)
LABS = *Gl. ambisporum* (Smith and Schenck)
LCLD = *Gl. caledonium* (Nicolson and Gerd.)
LCLR = *Gl. clarum* (Nicolson and Schenck)
LDMR = *Gl. dimorphicum* (Boyetchko and Tiwari)
LMST = *Gl. deserticola* (Trappe, Bloss and Merge)
LFSC = *Gl. fascicuiatum* (Thaxter)
LHOI = *Gl. hoi* (Brech and Trappe)
LOCT = *Gl. occultum* (Walker)
LHTS = *Gl. heterosporum* (Smith and Schenck)
LMSS = *Gl. mosseae* (Gerdemann and Trappe)
LMST = *Gl. muhisubstratum* (Mukerji, Bhatacharya and Tiwari)
LINR = *Gl. intraradices* (Smith and Schenck)
LPST = *Gl. pustulatum* (Koske, Frise, Walker and Dalpe)
GABD = *Gigaspora albida* (Schenck and Smith)
GRSA = G. *rosea* (Nicolson and Schenck)
CHTG = *Scutellospora heterogema* (Nicolson and Gerdemann)
CGRG = *Scutellospora gregeria* (Schenck and Nicolson)
LRBF = *Gl, rubiforme* (Almeda and Schenck)
LSNS = *GL sinosum* (Almeda and Schenck)

+++ = Highly intensive + = Less intensive - = Not observed

Species code: Perez and Schenck[32]
Schenck and Parez[33]
www.amf.phylogeny.com[34]

Material and Methods

The present experiments were conducted in the Botanical Garden, Department of Botany, Dr. H.S. Gour University, Sagar, MP. For such experiments 1 m^2 microplots were used. Collection of soil and root samples was done periodically from the rhizosphere after 40 DAS (seedling stage), 80 DAS (maturity stage) and 120 DAS (harvesting stage). Soil collected at 40 DAS was neither wet nor dry; at 80 DAS it was completely wet. At 120 DAS it was completely dry. Plants were uprooted with utmost care along with the wet soil from the depth of 4 to 6 cm. The rhizospheric soil was wrapped in plastic bags[16]. Every time soil samples brought to the laboratory and stored at 4°C until spore analysis.

Soil samples were sieved by wet sieving and decanting method[17] to determine the spore population. The roots were thoroughly washed in tap water and cut into 1 cm pieces, which were stained according to the method of Phillips and Hayman[18] for assessing mycorrhizal colonization.

Results

The data on root colonization and spore population with the number of AMF spp are presented in Table 1. The result shows that at 40 DAS, per cent colonization was 60%, spore population was 813 spores /100 gm^{-1} of soil and number of species was 15. Arbuscular diversity was recorded maximum. At 80 DAS per cent colonization was reduced to 29%, number of spores to 320 spores/100 gm^{-1} of soil and number of AMF species declined to 9. However, at 120 DAS percent colonization increased to 75%, spore population increased to the maximum of 996 spores/100 gm^{-1} of soil and the number of AMF species also increased to 22. Arbuscules were present in 40 DAS only, there after they were not observed. Vesicles started appearing in 80 DAS plants and increased in 120 DAS plants. In all total 24 AMF species were recorded. Among them *Glomus* and *Acaulospora* spp. were pre dominant in comparison to *Gigaspora* and *Scutellospora.*

Discussion

24 AMF species were found associated with roots of the paddy plants under investigation. Data presented in table 1 revealed that among 24 AMF species, 15 AMF species were recorded at 40 DAS, 9 AMF species at 80 DAS, and 22 AMF species at the time of harvesting (120 DAS). During this investigation, 8 AMF species were obtained at all the stages of cropping period. Occurrence of 8 AMF species throughout the cropping period suggests that these fungi can tolerate the conditions prevailing during the growth of the plants. Occurrence of *Gigaspora* only at 40 DAS and the formation of arbuscules in large quantity may also be corroborated. Disappearance of *Acaulospora scrobiculata* (Trappe), *Scutellospora gregeria* (Schenck and Nicolson), *S. heterogema* (Schenck and Gerdemann), *Glomus cladonium* (Nicolson and Gerd) and *G. intraradices* (Smith and Schenck) during the maturation phase *i.e.* 80 DAS, suggests that wet conditions became unfavourable for these AMF species. At this stage, anaerobiosis might have become detrimental to these fungi. Similar observations have also been made by Ilag[19]. However, Hayman[20] reported that the infectivity of AM fungal propagules decreases in wet and anoxic soils. Another important feature of waterlogged soil with high organic content is its attraction for wide array of soil organism enhancing the parasitic pressure on AM fungal spores[4]. The longer the soil is submerged under water, the more decrease in AM viability is noticed[19]. It is clearly evident that water stress condition was not suitable for better survival of AM fungi but AM fungi helped for better survival of plants in the adverse conditions.

From the results obtained, it is assumed that pH of soil might be playing some role in the disappearance and reappearance of AM fungi. However, Gai and Lui[21] have reported that pH above 7 favours the occurrence of *Glomus* species and pH below 7 favours the occurrence of *Acaulospora, Gigaspora* and *Scutellospora.* At 40 DAS the pH was 7.2, at 80 DAS it was 6.9 and at 120 DAS it was 7.5 (data not shown). Dominance of *Glomus* species at the time of harvesting may be corroborated with the high pH of soil. Schreiner and Koide[22] and Kapoor *et al.*[23] proposed that increased soil temperature stimulates AM colonization and spore production.

According to Morton and Bentivenga[24] most members of Glomineae and Gigasporineae form arbuscules. Maximum arbuscule development and root colonization takes place between 30°C-34°C. Vesicles develop to accumulate storage products in many VAM associations. Vesicles are initiated soon after the first arbuscules, but continue to develop when the arbuscules senesce. Vesicles are hyphal swellings in the root cortex that contain lipids in the cytoplasm. These may be inter- or intracellular. Vesicles can develop thick walls in older roots and may function as propagules[25]. According to Jalaluddin and Anwar[26] the quantity and the type of AM propaguales affected the dynamics of root infection which increased with the age of plants. In our recent findings we have reported dominance *of Glomus* and *Acaulospora* in the rhizosphere of rice varieties 'Basmati' and IR-36[27].

Therefore from this study it can be deduced that environmental condition influences the occurrence of AM fungi in the rhizosphere of rice. Dominance of *Glomus* in the forest and agriculture fields of Sagar region has already been reported.[28, 29, 30, 31].

Acknowledgement

Authors are thankful to Head, Department of Botany, Dr. H. S. Gour University, Sagar (M.P.) India for providing lab facilities. Deepak Vyas and Mahendra Kumar Mishra gratefully acknowledge UGC (India) for financial assistance.

References

1. Cardoso IM, Kuyper TW. Mycorrhizas and tropical soil fertility. *Agric Ecosyst Environ* 2006; **116** : 72-84.

2. Sanchez PA, Palm CA, Buol SW. Fertility capability soil classification a tool to help assess soil quality in the tropics. *Geoderma* 2003; **114** : 157-185.

3. Dalgaard T, Hutchings NJ, Porter JR. Agroecology of seedling and interdiscriplinarity. *Agric Ecosyst Environ* 2003; **100** :39-51.

4. Smith SE, Read RJ. Mycorrhizal symbiosis, Second Edition USA: Academic Press Inc, 1997.

5. Borowicz, VA. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? *Ecology* 2001; **82** : 3057-3068.

6. Redecker D, Kedner R, Graham L. Glomalean fungi from the Ordovician. *Science* 2000; **289** : 1920-1921.

7. Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kordos NL, Hedges SB. Molecular evidence for early colonization of land by fungi and plants. *Science* 2001; **293** : 1129-1133.

8. Judson OP, Nomork BB. Ancient asexual scandals. *Trends in Ecol Evol* 1996; **11** : A41-A46.

9. Olsson PA, Thirgstrup I, Jakobsen I, Baath F. Estimation of the biomass of arbuscualr mycorrhizal fungi in a Linseed field. *Soil Biol Biochem* 1999; **31** : 1879-1887.

10. Rillig MC. Arbuscular mycorrhizae, glomalian and soil aggregation. *Can J Soil Sci* 2004; **84** : 355-363.

11. Ryan MH, Graham JH. Is there a role for arbuscular mycorrhizal fungi in production agriculture? *Plant Soil* 2002; **244** : 263-271.

12. Cassman KG, Pingali PL. Intensification of irrigated rice systems: Learning from the past to meet future challenges. *Geojournal* 1995; **35** : 299-305.

13. De Datta SK. Principles and Practices of Rice Production. New York: John Wiley and Sons, 1981.

14. Sahrawat KL. Fertility and chemistry of rice soils in West Africa, State-of-the-art paper, Bouake, Ivory Coast: West Africa Rice Development Association, 1994.

15. Sahrawat KL. Fertility and organic matter in submerged rice soils. *Curr Sci* 2005; **88** : 735-739.

16. Gee GW, Bander JW. Particle Size analysis. In : Klute A. editor. Methods of soil analysis Part 1. Madison, WI: American Society of Agronomy. 1986.

17. Gerdemann JW, Nicolson TH. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans Br Mycol Soc* 1963; **46** : 235-244.

18. Phillips JM, Hayman DS. Improved Procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* 1970; **55** : 158-161.

19. Hag LL, Resales AM, Elazegui FA, Mew TW. Changes in the population of infective endomycorrhizal fungal in a rice-based cropping system. *Plant Soil* 1987; **103** : 67-73.

20. Hayman DS. Influences of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. *Phytopathol* 1982; **72** : 1119-1125.

21. Gai J, Liu R. Effects of soil factors on arbuscular mycorrhizae (AM) fungi around roots of wild plants. *Ying Yong Sheng Tai Xue Bao* 2003; **14** : 470-472.

22. Schreiner RP, Koide RT. Stimulation of vesicular-arbuscular mycorrhizal fungi by mycotrophic nonmycotrophic plant root sytsems. *Appl Environ Microbiol* 1993; **59** : 2750-2752.

23. Kapoor R, Giri B, Mukerji KG. Soil factors in relation to distribution and occurrence of VAM. In: Mukerji KG. *et al.* editors. Techniques in Mycorrhizal studies, Netherland; Kluwen Academic Publisher, 2002. pp.51 - 85.

24. Morton JB, Bentivenga SP. Levels of density in endomycorrhizal fungi (Glomales, Zygomycetes) and their role in defining taxonomic and nontaxonomic groups. *Plant Soil,* 1994; **159** : 47-59.

25. Biermann B. Linderman RG. Effect of container plant growth medium and fertilizer phosphorus on establishment and host growth response to vesicular-arbuscular mycorrhizae. *J Am Soc Hortic Sci* 1983; **108** : 962-971.

26. Jalaluddin M, Anwar QMK. VAM fungi in wheat and rice field. *Pak J Bot* 1991; **25** : 15-22.

27. Vyas D, Dubey A, Singh PK, Mishra MK. Distribution of VAM fungi in two different rice crop fields. *J Bot Soc* 2006; **41** : 160-170.

28. Dwivedi OP, Yadav RK, Vyas D, Vyas KM. Role of Potassium on the occurrence of VA mycorhizal spores in the rhizosphere of *Lantana* species. In: Jain PC. editor Microbiology and Biotechnology for Sustainable Development, New Delhi: CBS Publishers and Distributors, 2004. 248-253.

29. Vyas D. Diversity and distribution of VA mycorrhizal fungi in thirteen wheat cultivars.In: Bagyanarayana G, Bhadraiah B, Kumar IK. editors. Emerging trends in Mycology Plant Pathology and microbial biotechnology Hyderabad. B.S. Publications, 2005. 49-66.

30. Dubey A, Vyas D. Diversification on the occurrence of VAM fungi in rice *(Oryza* sativa). *J Bot Soc* 2005; **40**: 212-216.

31. Vyas D, Mishra MK, Singh PK, Soni PK. Mycorrhizal association in wheat grown in Sagar. *Ind Phytopath* 2006; **59** : 176-179.

32. Schenck NC, Perez Y. Manual for identification of AM fungi. Gainesville. USA: Synergistic Publication, 1987.

33. Perez Y, Schenck NC. A unique code for each species of VA mycorrhizal fungi. *Mycologia* 1990; **82** : 256-260.

34. www.amf.pylologeny.com

Proc. Nat. Acad. Sci. India, Sect. B, Vol. 78 Pt. I, 2008

Pachytene chromosome studies in fourteen accessions of *Carthamus* L.

ANJALI MALIK

Department of Microbiology, C.C.S. University, Meerut-250004, India.

Received February 12, 2007, Revised June 25, 2007, Accepted July 4, 2007

Abstract

Pachytene karyotypes in fourteen accessions, belonging to four species of the genus *Carthamus* were analyzed in detail for establishing the chromosome and karyotype polymorphism. The karyotype and heterochromatin distribution patterns reported in this study provide a foundation toward cytological characterization of the *Carthamus* genome. The chromosome size ranges between 502.74 μm to 979.97 μm. Variability within the individual chromosomes with respect to the size, shape and position of the centromere was found to be well marked. At pachytene, the pollen mother cells have 12 bivalents and prominent nueleolus. The accessions exhibited significant variability in their pachytene chromosome characteristics.

Key Words: *Carthamus*, pachytene karyotype, centromere

सारांश

गुणसूत्र एवम् गुणसूत्रीगठन बहुरूपता को देखने के लिये *कॉरथैमस* के चौदह प्रकारों की चार प्रजातियों का विस्तृत विश्लेषण किया गया है। प्रस्तुत अध्ययन में गुणसूत्रीगठन एवं विषमवर्णता के वितरण का विवरण *कॉरथैमस* जीनोम के कोशकीय लक्षणों को प्रदर्शित करता है। गुणसूत्रों के माप का क्षेत्र 502.74 माइक्रोमीटर से 979.79 माइक्रोमीटर के मध्य तक है। विशिष्ट गुणसूत्र अपने माप, आकार एवं स्थिति के सन्दर्भ में पूर्ण रूप से विभिन्नता प्रदर्शित करते है।

स्थूलपट्ट अवस्था में लघुबीजाणु मात्र कोशिका 12 युगली अथवा बाइवैलेण्ट तथा श्रेष्ठतम केन्द्रिका दर्शाती है।

सभी प्रकार अपने स्थूलपट्ट गुणसूत्रों में विख्यात विभिन्नतायें प्रदर्शित करते है।

सांकेतिक शब्द : *कार्थमस*, पैकीटीन गुणसूत्री गठन, सेन्ट्रोमियर।

Introduction

The genus *Carthamus* L., a member of the family Compositae has about 42 species with varying chromosome number 2n=20 to 2n=64. *Carthamus tinctorius* commonly called, as Safflower is the only cultivated species of this genus. The species is fast gaining importance as an oil crop. Earlier, this species was cultivated for extracting dye for food and cloths. Modern researches have shown that the Safflower oil can reduce coronary heart disease by lowering the cholesterol level. The somatic chromosome analysis of the genus *Carthamus* is very difficult due to poor stainability, stickiness and tendency of chromosomes to overlap at metaphase and diffused appearance of primary and secondary constrictions of the chromosomes. Usefulness of the pachytene stage of meiosis for studying detailed morphology of the chromosome was first emphasized by McClintock[1]. It is the stage of meiosis I, in which complete pairing of chromosomes and lining up of the chromomeres takes place and synapsis is at its climax. The pachytene analysis in accessions of the genus *Carthamus* has been conducted in the present study.

Material and Methods

Fourteen accessions belonging to four species of *Carthamus* were explored for the details of pachytene karyotype (Table 1). Young floral buds were collected and fixed in Carnoy's fluid II (6:3:1::absolute ethanol:chloroform:glacial acetic acid) for 24 hours and stored in 70% ethanol in refrigerator. Anthers were squashed and smeared in 1.5% aceto-carmine. All the observations and photomicrographs were taken from squashed temporary preparations. Only well spread pollen mother cells were considered for detailed studies. The pachytene chromosomes were analyzed from the photomicrographs using computerized Nikon Image Capturing system.

Details of karyotype were analyzed for (a). Total length of the chromosome of a complement (TLCC), (b). Length of long and short arms and the whole chromosome, (c). Arm's ratio (AR), (d). Total length of all short arms (TLSA), (e). Total length of all long arms (TLLA), (f). Centromeric index (ci), (g). Gradient index (GI), (h). Symmetry index (SI), (i), Total chromatin

length (TC1%) and (j). Percent chromomere per chromosome (CPC%) (Srivastava and Poornima)[2]

Arm's ratio, Ci, GI, SI, TC1%, relative length, and volume were worked out by using the formulae given below.

$$\text{Arm's ratio} = \frac{\text{Length of long arm of a chromosome}}{\text{Length of short arm of a chromosome}}$$

$$\text{Ci} = \frac{\text{Length of short arm of a chromosome}}{\text{Total length of the chromosome}} \times 100$$

$$\text{GI} = \frac{\text{Length of shortest chromosome of the complement}}{\text{Length of longest chromosome of the complement}} \times 100$$

$$\text{SI} = \frac{\text{Total length of all short arms}}{\text{Total length of all long arms}} \times 100$$

$$\text{TC1\%} = \frac{\text{Total length of a chromosome pair}}{\text{Total length of the gametophytic chromosome set}} \times 100$$

$$\text{CPC \%} = \frac{\text{Total amount of chromomere in long and short arm}}{\text{Total length of long and short arm}} \times 100$$

Table 1 - List of accessions/species used for pachytene chromosomes analyses.

S.No.	Lab Code	Species	Source Country
1	G-2	*C.glaucus*	China
2	L-l	*C.lanatus*	E.Germany
3	L-2	*C.lanatus*	E.Germany
4	L-4	*C.lanatus*	E. Germany
5	L-5	*C.lanatus*	E.Germany
6	L-6	*C.lanatus*	Belgium
7	L-7	*C.lanatus*	Portugal
8	P-4	*C.palaestinus*	Israel
9	P-6	*C.palaestinus*	Israel
10	T-2	*C.tinctorius*	Israel
11	T-10	*C.tinctorius*	Afghanistan
12	T-11	*C.tinctorius*	Afghanistan
13	T-17	*C. tinctorius*	China
14	T-24	*C. tinctorius*	China

Table 2 - Data related to pachytene karyotype of *Carthamus* accessions/species.

Code	Accessions	TLCC	TLLA	TLSA	GI	SI	TCL	KF
G-2	*C.glaucus*	599.53	327.66	271.87	30.36	82.97	215.43	KF=12D(m)+10E(m)+2E(m)
L-1	*C.lanatus*	561.15	292.40	268.75	28.57	91.91	320.34	KF=8D(M)+10E(m)+6E(M)
L-2	*C. lanatus*	502.74	299.71	203.03	20.69	67.74	221.02	KF=10D(m)+8E(sm)+8E(m)
L-4	*C.lanatus*	657.47	365.07	292.40	24.11	80.09	327.23	KF=10D(m)+14E(m)
L-5	*C. lanatus*	690.89	376.23	314.66	22.99	83.64	398.61	KF=16D(m)+2E(M)+4E(m)+2E(sm)
L-6	*C.lanatus*	979.97	532.77	447.20	32.92	83.94	329.38	KF=2B(m)+22D(m)
L-7	*C.lanatus*	832.48	463.54	368.94	11.65	79.59	350.88	KF=2C(m)+16D(m)+4E(m)+2E(sm)
P-4	*C.palaeslinus*	559.00	288.10	270.90	24.75	94.03	270.90	KF=6D(m)+4D(M)+8E(m)+6E{M)
P-6	*C.pataestinus*	723.27	398.42	324.85	22.04	81.53	331.96	KF=2C(m)4 14D(m)+6E(m)+2E(sm)
T-2	*C.tinctorius*	660.05	341.85	318.20	21.05	93.08	316.48	KF=14D(m)+10E(m)
T-10	*C.tinctorius*	645.00	357.33	287.67	51.91	80.51	386.57	KF=14D(m)+10E(m)
T-11	*C.linctorius*	863.80	447.45	416.35	44.19	93.05	378.83	KF=8D(m)+14D(M)+2E(m)
T-17	*C.tinctorius*	677.25	370.23	307.02	29.21	82.93	169.85	KF=14D(m)+10E(m)
T-24	*C.tinctorius*	515.70	303.71	211.99	20.69	69.80	344.43	KF=10D(m)+8E(sm)+6E(m)

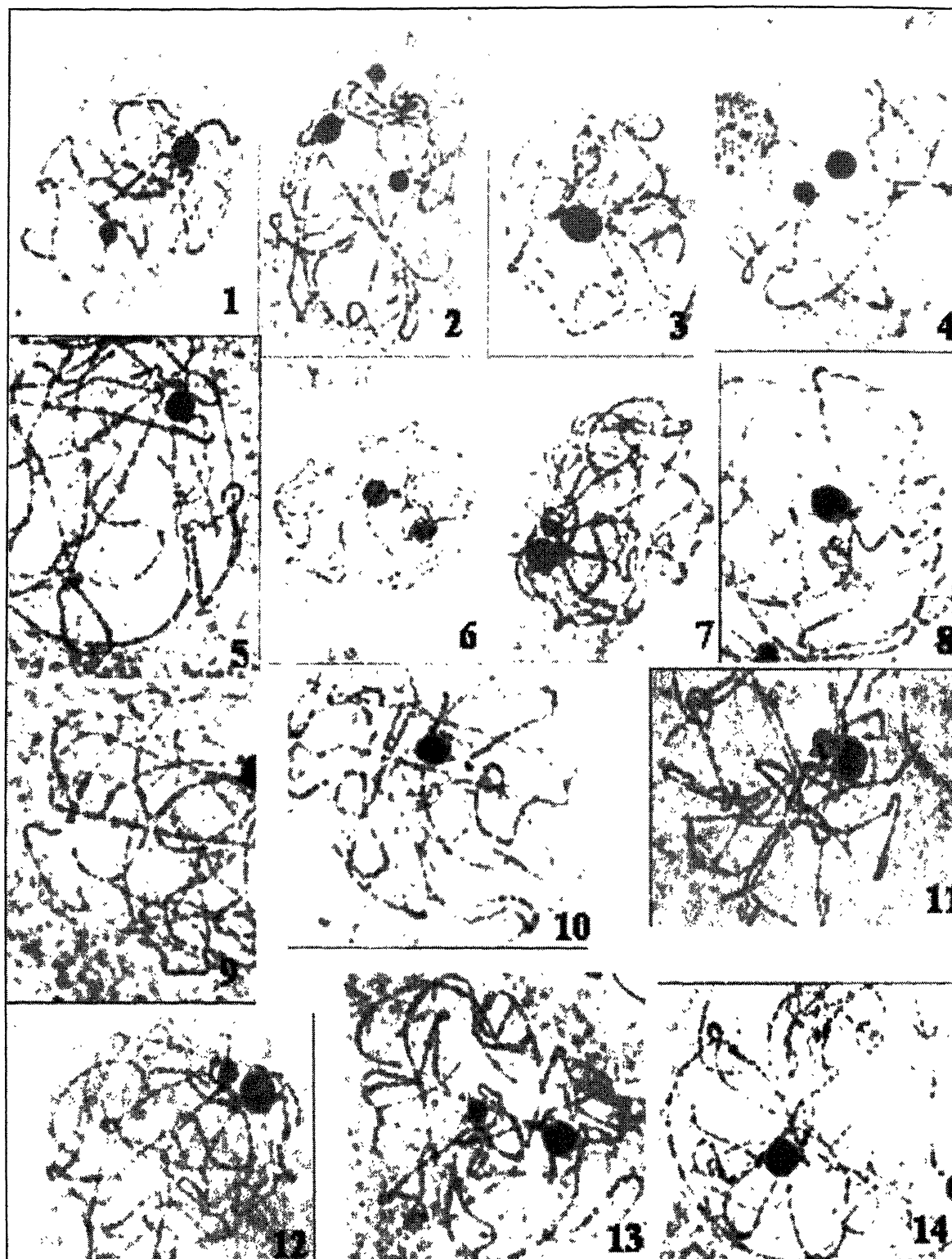



Fig. 1-14. Photomicrographs showing pachytene chromosomes in different *Carthamus accessions*. 1. G-2, 2. L-1, 3. L-2, 4. L-4, 5. L-5, 6. L-6, 7. L-7, 8. P-4, 9. P-6, 10. T-2, 11. T-10, 12. T-11, 13. T-17, 14. T-24.

Results and Discussion

At pachytene, the pollen mother cells have 12 bivalents and prominent nucleolus. The chromosomes are long and intertwined. For analysis of the pachytene karyotype, the parameters listed with material and methods were analyzed. Data pertaining to the pachytene karyotype analysis are listed in table 2. This table contains karyotype formulae based on pachytene analysis. The chromosome size ranges between 502.74 µm to 979.97 µm. The pachytene chromosomes of the complement were arranged on the basis of the total length into five types. A-E (A =250-200 µm, B = 200-150 µm, C =150-100 µm, D =100-50 µm, E =<50 µm). The pachytene chromosome were further divided into different categories as per Levan *et al.*[3] on the basis of arm ratio (M=1.0, nr=1.0-1.7, sm=1.7-3.0). Most of the pachytene chromosomes were of 'm' and 'M' type. However, 'sm' type chromosomes were also present in low frequency in many accessions.

Only selected photomicrographs of typical pollen mother cells showing pachytene chromosomes are presented in figures 1-14 because pollen mother cells were large. Total amount of chromomeres present in the total length of the long and short arms of the gametophytic set of pachytene chromosomes of all the fourteen accessions are graphically represented in figure 15.

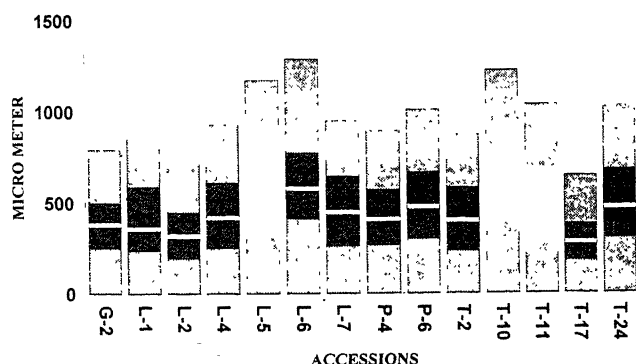



Fig. 15 - Graphical representation of total amount of chromomeres present in total length of long and short arms of the pachytene chromosomes in different accessions.

Cytogenetic studies of higher eukaryotic genomes are based on reliable and easy-to-use techniques for chromosome identification. The details of pachytene karyotype were analyzed using the parameters as per Srivastava and Purnima[2] and Srivastava and Kalara[4]. The somatic chromosome of the species of the genus *Carthamus* are difficult to resolve due to poor stainability, stickiness and tendency to overlap at metaphase. The centromeric portions and the secondary constrictions are not easily recognizable (Ashri and Knowles[5], Estilai and Knowlesr[6], Pillai *et al.*[7] Jayaramu and Chatterjii[8], Kumar[9]). Because of this the karyotype analyses in the genus *Carthamus* has been undertaken scantily in the past. Pachytene studies have an edge over somatic karyology for the purpose of chromosome identification. This is because at this stage chromosomes are relatively less condensed and therefore reveal their structural landmarks more easily. This karyotype is anchored by centromere-specific and chromosomal arm-specific cytological landmarks. The karyotype and heterochromatin distribution patterns reported in this study provide a foundation toward cytological characterization of the *Carthamus* genome. On account of these advantages, they have been employed for identification in a variety of angiosperms eg. Maize (McClintok[1], Rhoades and McClintock[10]), tomato (Barton[11], Rick and Barton[12]), rye (Lima-De-Faria[13]), barley (Sarvella *et* al.[14]), rice (Sen[15], Khush *et al.*[16]) and Castor (Paris *et al.*[17]) etc. The accessions differed significantly in the length of long and short arms of the same set and different sets, total length of long and short arms of a chromosome set and total amount of chromomeres present in a set of chromosome. The comparison of pachytene karyotype related to all these accessions indicates that all the accessions do differ in their pachytene karyotype. To the best of our knowledge the pachytene analysis in accessions/species of the genus *Carthamus* has been conducted for the first time. The length of the chromosome and distribution pattern of chromomeric segments exhibits remarkable differences between chromosomes of a complement and between the chromosome complements of different accessions of *Carthamus*.

Acknowledgement

The author is grateful to Prof. A. K. Srivastava Head, Department of Botany, C.C.S. University Meerut for his sincere guidance and advice; and for providing necessary facilities.

References

1. Mc Clintok B. Cytological observation of deficiencies involving known genes, translations and an inversion in *Zea-mays Miss Agric Exp Stat Bull* 1931; **163** : 1-30.
2. Srivastava A K, Purnima. Numerical and structural inconstancy in *Belamchanda chinemis* DC (Iridaceae). *Proc Indian Acad Sci* 1990; **100** : 205-210.

3. Levan A, Fredgra K, Samber AA Nomenclature for centromeric position on chromosomes. *Hereditas* 1964; **52**: 201-220.

4. Srivastava A K, Kalra R. Three dimensional analysis of karyotypes in *Carthamus*. *J Cytol Genet* 1996; **31(2)** : 139-144.

5. Ashri A, Knowles P F. Cytogenetics of safflower (*Carthamus L.*) species and their hybrids. *Agron J* 1960; **52(1)** : 11-17.

6. Estilai A, Knowles P F. Aneuploids in safflower. *Crop Sci* 1980; **20** : 516-518.

7. Pillai R S N, Kumar H, Singh RB. Karyotype analysis of safflower. *Crop Sci* 1981; **27** : 809-811.

8. Jayaramu M, Chatterji A K. Karyological studies on Indian wild safflower *Carthamus oxycantha* M.B. *Karyologia* 1986: **39** : 179-184.

9. Kumar H. Cytogenetics of safflower. In: Gupta P K, Ysuchiya T, editors. Chromosome engineering in plants-second Edition, Netherland: Elsevier Science Publishing Company, 1991; 251-278.

10. Rhoades M M, McClintock B. The Cytogenetics of maize. *Bot Rev J* 1935; **1** : 292-325.

11. Barton D W. Pachytene morphology of the tomato chromosome complement. *Amer J Bot* 1950; **37** : 639-643.

12. Rick C M, Barton D W. Cytological and genetical identification of the primary trisomics of the totmato. *Genetics* 1954; **39** : 640-644.

13. Lima-De-Faria A. Chromosome analysis of the chromosome complement of rye. *Chromosoma* 1952; **5** : 1-68.

14. Sarvella P, Homelgren J B, Nilan R A. Analysis of barley pachytene chromosome. *Nucleus* 1958; **1** : 183-204.

15. Sen S K. Cytogenetics of trisomics in rice. *Cytologia* 1965; **30** : 229-238.

16. Khush G S, Singh R J, Sur S C, Librojo A L. Primary trisomics of rice: Origin, morphology, cytology and uses in linkage mapping. *Genetics* 1984; **107** : 141-363.

17. Paris H S, Shifriss O, Jelenkovic G J. Idiograms of *Ricinus communi. Hered* 1935; **69** : 191-196.

Arbuscular mycorrhizal colonization status and growth of *Acacia catechu* Wills. seedlings under pesticide application

MANOJ TIWARI, ARCHANA TIWARI and NEERJA PANDE

Department of Botany, Kumaun University, Nainital, Uttaranchal, India.

Received October 10, 2005, Revised May 23, 2007, Accepted May 25, 2007

Abstract

Arbuscular mycorrhizal (AM) fungi and their effect on growth of *Acacia catechu* seedlings was studied in relation to pesticide application. Three broad spectrum pesticides viz: a fungicide, a nematicide and an insecticide were applied in all possible combinations to remove maximum possible number of target organisms from the soil. When applied separately at selected doses, these pesticides show no effect on AM status and dry weight of seedlings, and had negative effect in combination.

Key words : AM fungi, soil biota, pesticide, *Acacia catechu.*

सारांश

आकेसिया कटेचू के पौध की बढ़त पर कीटनाशक रसायन के संदर्भ में आरबसक्यूलर माइकोराइज़ल (A.M.) फफूंद के प्रभाव का अध्ययन किया गया। तीन विस्तृत वर्णक्रम के कीटनाशक रसायनों को, जिनमें एक फफूंदनाशक, एक निमैटोड़नाशक तथा एक कीटनाशक था, विभिन्न संयोजनों में मिट्टी में इस उद्देश्य से मिलाया गया कि अधिकांश लक्षित फफूंद हटाये जा सकें। उन्हें चयनित खुराकों में अलग अलग प्रयोग में लाने पर A.M. की स्थिति तथा पौध के शुष्क भार पर कोई प्रभाव नहीं दिखा, परन्तु, संयोजित अवस्था में इनका प्रभाव नकारात्मक रहा।

सांकेतिक शब्द : ए0एम0 कवक, मृदा जैविकी, कीट नाशक, *अकेसिया कटेचू*।

Introduction

One of the most important, but least understood aspect is ecological functioning of mycorrhiza [1-5]. This symbiosis is established in presence of soil organisms that flourish on root exudates, peeled off tissues and cells of their host root[6]. The quantitative and qualitative nature of these organisms in the rhizosphere is related to many environmental and physiological factors[7, 8]. Mycorrhizal fungi interact with these organisms either by direct contact between hyphae of mycorrhizal fungi and these organisms or modification of environment by any one organism by contact between one organism and the product of other[9-11]. Sometimes, these interactions are critical for the production of healthy seedlings of tree species in nursery[12-15]. In the present work AM fungal colonization of *Acacia catechu* is worked out in relation to pesticide application.

Material and Methods

The soil used in the study was collected from a natural stand of *Acacia catechu* Wills Var. Khair, forest at Haldwani, India. The soil (moist clayey loam with pH 6.2, WHC 40%, organic carbon 2.23%, total nitrogen 0.14% and total phosphorus 0.07%) was sieved through 4 mm sieve and mixed with washed white sand in 1:1 (v/v) ratio.

A. catechu seeds, collected from a single tree of the natural forest stand, were soaked in distilled water for 24 h. The germinated seeds were transferred in polybags (capacity 8 kg dry soil) containing sterilized or unsterilized soil : sand mixture. The steam sterilization was done for two consecutive days at 30 lb (120°C) for an hour to eliminate native population of soil organisms. Half of the polybags with sterilized mixture were inoculated with indigenous AM spores (*Glomus* spp.), collected from natural stand soil of *A. catechu*[16], at inoculum density equal to unsterilized mixture (~1000 spore/polybag) just before filling. Two seedlings were placed in each polybag containing 8 kg of soil, one for plant performance estimation and other for mycorrhizal performance.

Three broad spectrum pesticides viz: a fungicide [Captan 75% (100 mg l^{-1})], a nematicide [Carbafuran

3G (115 mg l^{-1})] and an insecticide [Malathion 50EC (1 mg l^{-1})] were applied to polybags just after seed transfer in all possible combinations, viz. fungicide (F), nematicide (N), insecticide (I), FN, NI, FI, FNI, O (control, no pesticide), creating a total of 24 (3x8) possible treatments with 5 replicates in each. Nematode, micro arthropod and fungal populations in soil: sand mixture were determined in unsterilized soil before pesticide application and at the end of the experiment to test the effectiveness of pesticides to remove target organisms.

The polybags were kept in glasshouse at 25±5°C, 75±10% RH. On alternate days each polybag was watered into 200 ml of autoclaved water. Plants were harvested after 12 months from the date of germination of seeds. Observations were recorded for root, shoot and seedling, both fresh and dry weight (48h at 60°C) and the seedling height. Fifty root segments were randomly selected to observe % infection and % colonization of AM fungi by staining procedure and Grid line intersect method of assessment given by Phillip and Hayman[17] modified by Kormanic and McGraw[18]. Mycorrhizal occupancy was calculated as infection x % colonization)/100.

Differences between means were tested using Tukey's honestly significant *t* test or Mann-Whitney *U* test. All the statistics were performed using SYSTAT procedures[19].

Results

Mycorrhizal colonization (MC) of seedlings in sterilized soil with *Glomus* spp. and unsterilized soil was significantly different ($p<0.05$). It correlated significantly with seedling's dry weight ($r=0.73$, $p<0.001$), in sterilized soil with AM inoculation, application of pesticides significantly reduced MC of seedlings over that of the control, being maximum for the one receiving three pesticides together. Seedlings receiving two pesticides together showed lesser MC than the one receiving single pesticide but the difference was not significant ($p>0.05$). In unsterilized soil, least MC (17%) was observed on seedlings in control treatment that had all soil organisms ($p<0.05$). Seedlings in fungicide treatments, alone or in combination, showed lesser increment in MC (41% to 135%) in comparison with the other pesticide treatments without fungicide (141% to 194%) over that of the control (all $p<0.05$) (Fig. 1).

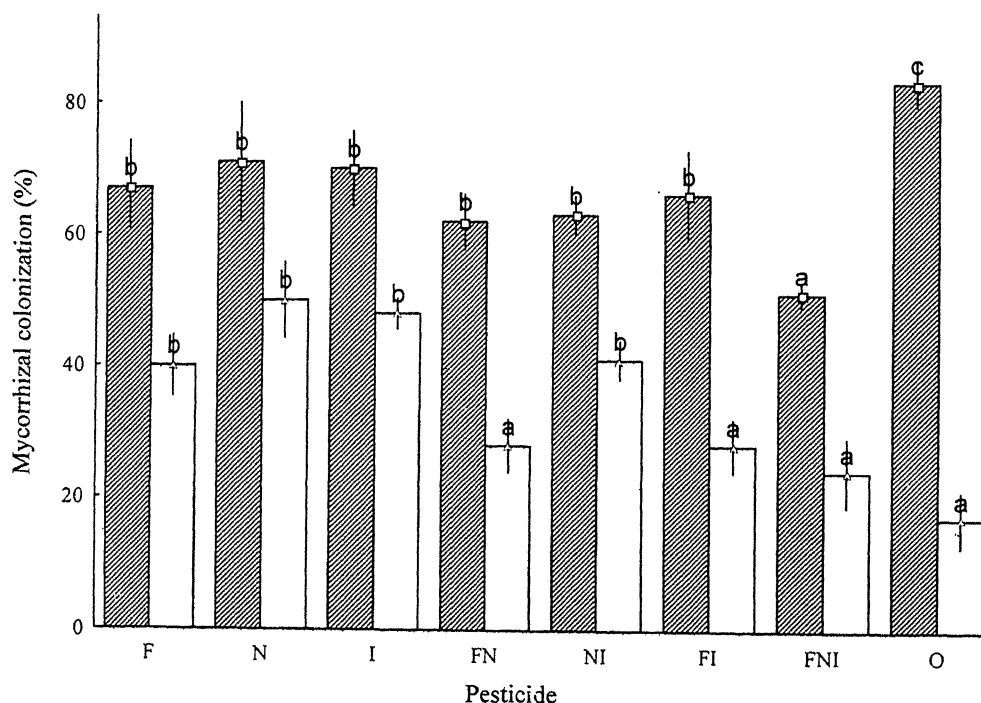



Fig. 1 - Mycorrhizal status of 12-months old *A. catechu* seedlings as affected by pesticide application in sterilized + AM (▨) and unsterilized (□) soils. Values with different letters at the top of columns for one soil are significantly different (Tukey's honestly significant *t* test, p = 0.05). Values are Mean ± 1SE (n = 5).

Fresh and dry weight of all plant parts were significantly correlated (root r=0.83, shoot r=0.79, seedling r=0.78; all p<0.001). Dry weights of root and shoot were also significantly correlated (r=0.87, p<0.001), and consequently both were highly correlated (autocorrelation) with seedling's dry weight suggesting that none of the pesticide treatment and AM inoculation affected functional equilibrium between root and shoot, which varies between 0.74 to 0.93. Seedling height also correlated significantly with seedling dry weight (r=0.90, p<0.001). In view of these highly significant correlations, only seedling dry weight was analyzed further as a general indicator of plant performance.

In all pesticide treatments, seedlings in sterilized soil with AM inoculation had higher dry weight than that in unsterilized soil (1.5% to 63.2%, Mann- Whitney *U* test; p<0.001). AM association with seedlings increased their dry weight, causing 42% to 129% and 0% to 126% increment in sterilized and unsterilized soils (Mann-Whitney *U* test; both p<0.001) respectively, at all pesticide treatments, except control. In control treatment (no pesticide), seedling dry weight in sterilized soil without AM inoculation and unsterilized soil were not significantly different (Mann-Whitney *U* test; p>0.05), showing that the organisms present in unsterilized soil severely curtailed the positive effect of AM association on seedling growth (Fig. 2).

In sterilized soil, seedlings receiving more than one pesticide observed greater reduction (significant or not) in dry weight than the one receiving single pesticide. Maximum reduction was observed by seedlings receiving three pesticides together, i.e. FNI (11 % with and 42% without AM inoculation), but reduction from control was significant in sterilized soil without AM inoculation only (p<0.05). This shows that AM infection on seedling root provides some protection against toxic effect of pesticides on seedling performance (Fig. 2).

Application of pesticides significantly increased seedling dry weight in unsterilized soil over that of the control, being maximum for seedlings receiving nematicide (47.4% to 54.7%), either alone or in combination with other pesticides (all p<0.05) and minimum for the one receiving fungicide (2.1%, p>0.05) (Fig. 2).

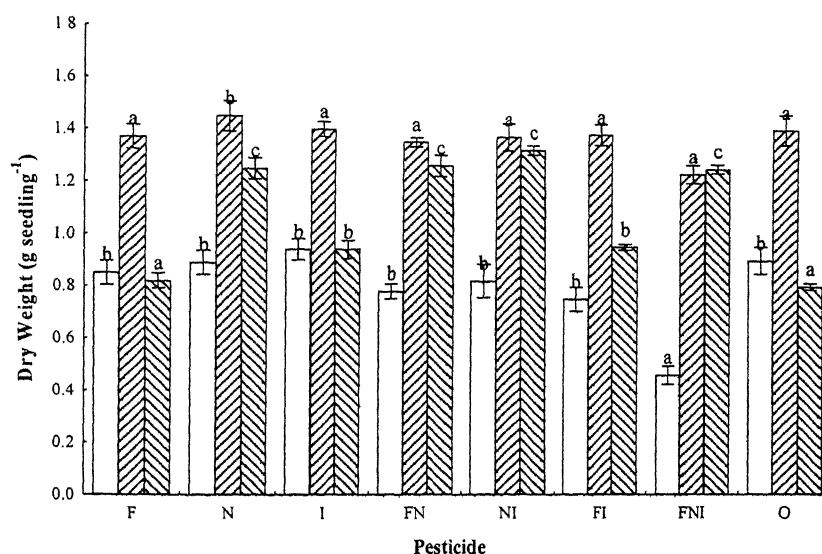



Fig. 2 - Average biomass of 12-months old *A. catechu* seedlings as affected by pesticide application in sterilized (▭), sterlized + AM (▨) and unsterlized (▧) soils. Values with different letters at the top of columns for one soil are significantly different (Tukey's honestly significant *t* test, p = 0.05). Values are Means ± 1SE (n = 5).

Discussion

The main objective of the experiment was to study the effect of interactions between AM fungi and other soil organisms on plant performance. Thus, only pesticides with neutral or positive effects on mycorrhizal performance were selected for present experiment. The three broad spectrum pesticides and their doses were selected with the idea of removing the highest possible number of organisms from each target group with little or no effect on seedling or AM status. Although successful in controlling pests and pathogens, the application of some pesticides may also result in indiscriminate killing of beneficial soil organisms[20-23]. Negative effects of these pesticides on AM at the doses applied in the present experiment have neither been reported[24-25] nor did they appear in this study, when applied separately, but the combination of pesticides might well exceed the threshold for AM fungi and in turn negatively affected mycorrhizal colonization and host growth. This dose dependent effect of combination of pesticides could well explain the observed negative effect of total combination of pesticides in sterilized soil (where soil biota had already been reduced).

The largest increment in dry weight of *A. catechu* seedlings have been obtained by the use of nematicide. Harmful effects of plant parasitic nematodes and possibly of fungivorous nematodes that graze on the mycorrhizal mycelium[13] play a key role in the availability of insoluble nutrients in the soil. It can, therefore, be a serious problem for the growth of *A. catechu* and other plants in general. Application of insecticide eliminated both root herbivorous and fungivorous micro arthropods but it has resulted in only slight improvement in AM status and performance of *A. catechu* seedlings. Microarthropods are efficient grazer of mycorrhizal fungi[12, 13] and reduce the infection rate between the mycorrhizal fungi and the plant root[15]. Removal of fungi from the soil, by fungicide application, had no effect on seedling and AM performance. But eliminating fungi might shift the balance between root herbivorous and fungivorous biota, which might also affect plant performance.

These interactions of AM fungi and soil biota are of relevance because they can affect plant establishment, development and nutrient acquisition. This experiment sheds light on the kind of interactions that occur between AM fungi and soil organisms and its resulting effect on plant growth. However, more studies are needed to evaluate plant responses to specific interaction. The extent of our knowledge of details of these relationships will significantly affect any consideration of interactions of various soil organisms with mycorrhiza.

Reference

1. Plencette C, Clermont-Dauphin C, Meynard JM, Fortin JA. Managing arbuscular mycorrhizal fungi in cropping systems. *Canadian J Plant Sciences* 2005; **85**: 31-40.
2. Thangaswamy S, Padmanabhan C. Arbuscular mycorrhizae: A diverse personality. *J Central Europian Agriculture* 2006; 7 : 349-358.
3. Wigand C, Stevenson JC. The presence and possible ecological significance of mycorrhizae of the submersed macrophyte, *Vallisneria americana. Estuaries* 1994; **17**: 206-215.
4. Menkis A. Root Associated Fungi of Conifer Seedlings and Their Role in Afforestation of Agricultural Land. Ph. D. Thesis. Swedish University of Agricultural Sciences, Uppsala, 2005.
5. Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM. How plants communicate using the underground information superhighway. *Trends in Plant Science* 2004; **9** : 26-32.
6. Marschner H, Dell B. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* 1994; **159** : 89-102.
7. Ingrid MA, Pal AO, Soderstrom B. Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. *New Phytologist* 2002; **155** : 173-182.
8. Strzelczyk E, Kampert M, Pachlewski R. The influence of pH and temperature on ethylene production by mycorrhizal fungi of pine. *Mycorrhiza* 1994; **4** : 193-196.
9. Garbaye J. Biological interactions in the mycorrhizosphere. *Experientia* 1991; **47** : 370-375.
10. Abbott LK, Robson AD. Factors influencing the occurrence of VA mycorrhizal fungi. *Agriculture, Ecosystems and Environment* 1991; **35** : 121-150.
11. Ek H, Sjogren M, Arnebrandt K, Sodrstrom B. Extramatrical mycelial growth, biomass allocation and nitrogen uptake in ectomycorrhizal systems in response to collembolan grazing. *Applied Soil Ecology* 1994; **1**: 155-169.
12. McGonigle TP, Fitter AH. Ecological consequences of arthropod grazing on VA mycorrhizal fungi. *Proc R Soc Edinburgh* 1990; **946** : 25-32.

13. Azcon AC, Barea JM. Interaction between mycorrhizal fungi and other rhizosphere microorganisms. In: Allen MF, editor. Mycorrhizal functioning: an integrative plant-fungal process. The Netherlands: Chapman and Hall, 1992; 163-198.

14. Smith SE, Read DJ. Mycorrhizal symbiosis. London: Academic Press, 1997.

15. Harris KK, Boerner REJ. Effects of below-ground grazing by *Collembola* on growth, mycorrhizal infection and P uptake by *Geranium robertianum*. *Plant and Soil* 1990; **129** : 203-210.

16. Pacioni G. Wet-seiving and Decanting techniques for the extraction of spores of Vesicular-arbuscular fungi. In: Norris RJ, Read DJ, Verma AK, editors. Methods in Microbiology, vol. 24, London: Academic Press, 1992; 317-322.

17. Phillips JM, Hayman DS. Improved procedures for cleaning roots and staining parasites and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans British Mycological Soc* 1970; **5** : p. 158.

18. Kormanic PP, McGraw AC. Quantification of vesicular-arbuscular mycorrhizal fungi in plant root. In: Schenck NC, editor. Methods and Principles of Mycorrhizal Research. St. Paul: The American Phytopathological Society, 1982. p. 37.

19. Wilkinson L. The system for statistics. SYSTAT, Illinois, USA: Evanston, 1990.

20. Menendez A, Martinez A, Chiocchio V, Venedikian N, Ocampo JA, Godeas A. Influence of the insecticide dimethoate on arbuscular mycorrhizal colonization and growth in soybean plants. *International Microbiology* 1999; **2** : 43-45.

21. Endlweber K, Schadler M, Scheu S. Effects of foliar and soil insecticide applications on the collembolan community of an early set-aside arable field. *Applied Soil Ecology* 2006; **31** : 136-146.

22. Manjunath A, Bagyaraj DJ. Effect of fungicides on mycorrhizal colonization and growth of onion. *Plant and Soil* 1984; **80** : 147-150.

23. Muchovej JJ, Muchovej RMC, Goncalves EJ. Effect of kind and method of fungicidal treatment of bean seed on infections by the VA mycorrhizal fungus *Glomus macrocarpum* and by the pathogenic fungus *Fusarium solani*. 2. Temporal-spatial relationships. *Plant and Soil* 1991; **132** : 47-51.

24. Sreenivasa MN, Bagyaraj DJ. Use of pesticides for mass production of vesicular arbuscular mycorrhizal inoculum. *Plant and Soil* 1989; **119** : p. 127.

25. Jalali BL. Side effects of pesticides on mycorrhizal system-An overview. In: Jalali BL, Chand H, editors. Current Trends in Mycorrhizal Research. Hissar, India: Haryana Agriculture University Press, 1990; 35-36.

Proc. Nat. Acad. Sci. India, Sect. B, Vol. 78 Pt. I, 2008

Eventide blooming, insect pollination, low fruiting and seeding in the strychnine tree, *Strychnos nux-vomica* Linn.

J.B. ATLURI, S.P.V. RAMANA and C. SUBBA REDDI*

*Department of Botany, *Environmental Sciences, Andhra University, Visakhapatnam-530003, India.*

Received March 29, 2007, Revised August 14, 2007, Accepted August 30, 2007

Abstract

Natural populations of *Strychnos nux-vomica* Linn. in Eastern Ghats region produce heavy bloom during February - April. The flowers open in the evening hours during 1500h- 2000h, with a peak at 1600h - 1800 h. A syrphid fly *Sphaerophoria indiana* and three bee species *Xylocopa latipes* (juveniles), *Apis cerana indica* and *Trigona iridipennis* are the pollinators. *S. nux-vomica* seems to have adapted a mixed mating system with cryptic self-incompatibility. Fruits mature over a 10-11 month period and mature fruit set is very low i.e. 3.6%. There is heavy damage to flowers mostly by sooty mould *Capnodium* sp. and also by aphid infestation and egg-laying by syrphid fly. Only 3-4 seeds develop out of the 12-13 ovules in the ovary. The limited seediness of fruit might be of ecological advantage to *S. nux-vomica* since its fruit gets dispersed through water.

Key words : *Strychnos nux-vomica*, pollination, fruiting, syrphid fly, *Sphaerophoria indiana*.

सारांश

भारत के पूर्वी घाटों पर प्राकृतिक व्यवस्था में पाये जाने वाले पौधे, *स्ट्रिक्नास नक्स–वोमिका* लीनियस में फ़रवरी से अप्रैल तक के महीनों में अत्यधिक पुष्प खिलते हैं। पुष्पों के खिलने का सामान्य समय संध्या बेला में 1500 घंटे से 2000 घंटे तक का होता है, जिसमें 1600 घंटे से 1800 घंटे के मध्य यह प्रक्रिया अपने चरम पर होती है। पुष्पों में परागण की प्रक्रिया प्रमुखतः *स्फेरोफ़ेरिया इंडियाना* तथा मधुमक्खियों की तीन प्रजातियों, *ज़ाइलोकोपा लैटीसेप्स (नवजात), एपिस सेराना इंडिका* तथा *ट्राइगोना इरीडीपेनिस* द्वारा संपादित होती है। *एस नक्स वोमिका* में संदेहास्पद स्वअसंगत संभोगी के साथ मिश्रित संमोगी व्यवस्था आत्मसात होती जान पड़ती है। लगभग 3.6% फल, 10 से 11 महीनों के अंतराल में पक कर तैयार होते हैं। पुष्पों को अत्यधिक हानि सूटी मोल्ड *कैप्नोडियम* स्पीसीज, एफ़िड तथा सिरफ़िड मक्खियों के अंडों से होती है। अंडाशय में मौजूद 12–13 अंडाणु केवल 3 से 4 बीज ही बना पाते हैं। इसके फलों का स्थानान्तरण जल द्वारा होता है।

सांकेतिक शब्द : *स्ट्रिक्नास नक्स–वोमिका*, पराग निषेचन, फलना, सिरफ़िड मक्खी, *स्फेरोफ़ोरिया इन्डियाना*।

Introduction

In recent years there has been an increased focus on minor forest produce as a source of financial incentive to the local tribal communities so that they can be weaned from associating with the illegal timber merchants and thus preserve the natural forests. Amongst a great variety of minor forest products, the seeds of many tree species are of very high economic value. The strychnine tree *Strychnos nux-vomica* Linn. (Loganiaceae) is one among such valuable tree species. It is a native of south-east Asia, especially India and Myanmar, and is distributed throughout tropical India. The seeds are a source of two important and strongly toxic alkaloids: strychnine and brucine, which are used in indigenous medicine as a tonic and stimulant. In India, the local tribal communites collect the seeds from the wild grown plants. The seeds are freed from the heavy bitter pulp of the ripened fruits, washed thoroughly with water, and dried in the sun. About 15,000 tonnes of seeds are thus collected annually and sold in the domestic and export market. In the present study on the reproductive ecology of economically important tree species of Eastern Ghats forests, the authors observed that fruit and seed output of the strychnine tree varied much from year to year and some trees even totally failed to reproduce. With a view to find out the probable causes for such variation in reproduction, the authors studied the blooming phenology, floral dynamics, functional mating system, mode of pollination, flower foragers, fruiting and seeding patterns.

Material and Methods

Observations were made using the trees of *Strychnos nux-vomica* growing as wild in the natural forests at Govindapuram in the Eastern Ghat region of East

Table 1 - Results of hand - pollination tests of different breeding systems in *S. nux-vomica*

Type of breeding system	No. of flowers hand-pollinated	No. of flowers set	Fruit set (%)
Apomixis	20	0	0
Autogamy			
Spontaneous	20	0	0
Manipulated	20	10	50
Geitonogamy	20	12	60
Xenogamy	20	18	90

Godavari district in Andhra Pradesh. The course of blooming of 10 different trees at the study site was recorded through weekly visits or when needed by continuous stay. Flower opening pattern on daily and hourly basis was registered for 20 tagged inflorescences distributed on five trees. The flowers which opened each hour in a day were scored and removed to avoid their recounting the next hour. Mean pollen number per anther was determined using 10 mature anthers following Subba Reddi and Reddi[1]. After finding out the mean number of ovules in an ovary, pollen-ovule ratio was calculated as per Cruden[2]. Relative performance of auto-, geitono-and xenogamy, and apomixis were studied. In each treatment, 20 flowers were used. Spontaneous autogamy (autonomous selfing) was also tested by enclosing the matured inflorescences with paper bags. Fruit set in each treatment was recorded. The number of mature fruits produced per inflorescence in open pollination and the number of mature seeds per ripened fruit was counted. Pollen grains germinated well in 20% sucrose solution, and using the same concentration, pollen germination percentage in hanging drop was recorded at different intervals. Stigma receptivity period was assessed through visual means and hand-pollinations. Receptive stigmas were turgid, viscid and shiny. Such stigmas were divided into batches of five each, and hand-pollinated with fresh xeno-pollen as it proved to be more compatible, at 2-hourly intervals, and then bagged. The ability of these stigmas to produce fruit was taken to indicate their receptivity period. Flower visitors were observed through out the day and night. Their activity pattern including foraging speed in terms of number of flowers visited per unit time and the time (sec.) spent foraging at a flower, and relative frequency were recorded using stop-watch and observing the visitors as long as they were in sight on a selected branch of the tree.

Results

The plants are deciduous, with leaf fall occurring in the cold season at the end of December. As the cold season recedes and the summer begins, at around the first week of February, new leaves unfold. Blooming progresses through March and terminates by the end of April. Blooming is heavy with peak bloom spreading over 30 days from mid-February to mid-March, and the total blooming sustaining over a period of 61-67 days. Inflorescences are borne at the tips of short branchlets. They are cymose with flowers arranged closely giving the clustered appearance. They are oriented at an angle of 45° to the inflorescence axis. Flowers per inflorescence vary in number from 20-38 (av. 28). They exhibit a skewed distribution (Fig. 1). They mature and open in succession daily over a period of 5-8 days, with higher frequency on d_2-d_4.

Flowers open in the evening hours from 1500 to 2000 h under the prevailing temperatures of 28°C-35°C and RH of 70%-75%. Flower opening is maximum at 1600 h-1800 h (68%). Anthers dehisce by longitudinal slits within 30 minutes of flower opening. Flowers are greenish-white, 6-7 mm long, hermaphroditic, actinomorphic and homogamous (Fig. 2 A). They are nectariferous, with nectar in traces in opened flowers. Calyx has five green sepals. Corolla is in the form of a cylindrical tube with pubescence at the base. The five petals are greenish-white spreading out flat. Stamens are five with short filaments, inserted in the throat of the corolla alternating with the lobes. Anthers are basifixed, positioned a little above the corolla mouth. They are pale cream in colour, dithecous and introrse. Style projects a little beyond the corolla mouth and the capitate stigma is almost at par with the position of anthers. Ovary is bicarpellary, each carpel containing 12-13 ovules on axile placentation. Pollen production per an-

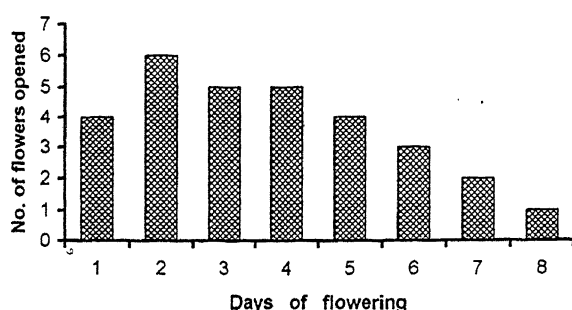



Fig. 1 - *Strychnos nux-vomica*-inflorescence blooming phenology.

ther is 1300-1600 (av. 1400), and per flower it averages 7000 grains. Pollen grains are smooth, light brown, spheroidal, tricolpate and 32-36 (33±2) µm in diameter. While the pollen/ovule ratio of 280:1 is indicative of facultative autogamy, hand-pollination tests gave a fruiting of 50%, 60% and 90% in auto-, geitono-, and xeno-gamy respectively (Table 1). Tests for apomixis and spontaneous autogamy are negative.

Pollen viability indicated by germination percentage of fresh pollen in 20% sucrose solution was 100%. It declined drastically with storage life of pollen, as it was 50% up to 8 h. The fresh xeno-pollen deposited on fresh stigma surfaces yielded 90% fruit set, 4-h old pollen gave 50% and the pollen stored for longer time gave < 20% fruit set. Stigma surfaces are receptive at flower opening time as indicated by their freshness and viscidity. Stigmas up to the age of 4 hours gave 90% fruit set after hand-pollination with fresh xeno-pollen, the 8-hold ones gave 30% fruit set. Thus, both pollen viability and stigma receptivity data suggest that fruit set in outcrossing could be around 90% if xenogamy is accomplished during the first 4-6 hours of flower life. Flower life lasts for 24h-30h, after which the corolla along with anthers drops down. The fallen corollas form a dense mat below the canopy, which gives the estimate of the tree's large flower biomass.

Natural fruiting varied between 20%-40% in different individual plants, the big trees bearing relatively more fruits. Interestingly the mature fruits per inflorescence were only 1-3. Inflorescences bearing a single fruit were more (79%) (Fig. 2 B) than those with 2 (19%) and 3 fruits (2%). On the whole, the production of mature fruits was about 3.6%. Fruits mature over a period of 10-11 months, and the normal fruit size reaches 6-10 cm in diameter. However in big trees the fruit size ranged from 12-18 cm in diameter. The number of fully developed seeds per fruit were 3-4 (ca.28%). The ash grey coloured seeds were compressed and disc-shaped, measuring 2.2 ± 0.2 cm across.

There was considerable damage of flowers due to aphid infestation and the consequent attraction and development of the sooty mould fungus *Capnodium* sp. Most of the inflorescences turned black and dried up (Fig. 2B) bearing masses of black spores of the fungus as revealed in the scrapings of the inflorescence. This sooty mould is common on many fruit trees in India[3]. A syrphid fly *Sphaerophoria indiana,* the larvae of which are aphidophagous, laid eggs on the aphid infested leaves and flower buds which did not develop further. Some trees did not show much of aphid infestation. They were visited by the syrphid fly for the collection of pollen grains. Young fruits are consumed and damaged by striped squirrels.

The pollinator exclusion experiments showed that *Strychnos nux-vomica,* under natural conditions, does not produce seeds either by apomixis or by spontaneous autogamy. It is thus inferred that *S. nux-vomica* strongly needs pollen vectors for accomplishing pollination. Three bee species including the juveniles of the carpenter bee *Xylocopa latipes,* the open nested Indian honeybee *Apis cerana indica,* and the stingless or dammar bee *Trigona iridipennis* and the syrphid fly *Spaerophoria indiana* (Fig. 2C) regularly visited the flowers. All the foragers appeared from flower opening time (1500 h) and continued up to 1800 h. The stingless bee and syrphid fly collect only the pollen grains but the others collect both pollen and nectar. A large number of syrphid flies seen hovering and foraging at the flowers undergo egg laying. Pollen consumption improves their egg laying ability. Their larvae feed on aphids[4]. The number of flowers visited per minute by each visitor species are 5-14 for *X. latipes* 3-12 for *A. cerana indica* 3-4 for *T. iridipennis* and 4-6 for *S. indiana.* The mean time spent at a flower by the respective foragers is 14, 4, 11, 8 seconds, and the number of loose pollen grains carried on the body respectively are 136, 60, 27 and 25. While foraging at the flowers, the four anthophilous insects come into contact with anthers and stigma located slightly above the rim of corolla tube. Pollen grains received on legs, ventral surfaces, head, and proboscis of the insect were transferred to the stigma surfaces either of the same flower or other flowers in the same inflorescence or the other inflorescences on the same

Fig. 2 - *Strychnos nux-vomica* : Photographs showing the inflorescence and flower top with spread out petals (A); Mature fruit of previous year, and *Capnodium* damaged inflorescences of the current year (B); and the syrphid fly *Sphaerophoria indiana* foraging at the flower (C).

plant or other conspecific plants resulting in self- as well as cross-pollination. The four visitor species were found to make inter-tree flights also. Night time observations showed no insect visits on the flowers.

Discussion

The observed flowering season of *Strychnos nux-vomica* is in conformity with the general dry season flowering of tree species in South India[5]. Daily flowering occurring in the evening hours between 1600-1800 h appears interesting. The flowers are not showy and attractive. However, by virtue of their aggregation forming inflorescences of considerable size, they become rather conspicuous. Their mild sweet odour might act as a close-in attractant to entice landing of flower visitors. Although open flowers are available through the dark hours of the succeeding night, there were no flower visitors at night. Probably the traces of nectar in the flowers may not be sufficient to support the nocturnal visitors[6]. The syrphid fly and the three bee species freely contacted the anthers and the stigma while foraging at the flowers. The loose pollen recovered from their body washings and the stigma pollen loads after their visits suggest that they are effective in mediating pollination. The larger floral display, apart from providing a larger signal to attract pollinators, insures that the pollinator visit rate will be sufficient for adequate pollination. Extended flowering season creates an opportunity to the visitors to pickup pollen on more occasions thus increasing the chances for the occurrence of out-crossing[7,8]. Thus the genetic diversity of seed progeny may be enhanced by larger floral display[9].

The number of fruits produced is very low compared to the quantity of flowers, but is close to the average fruit set of 22.1% reported in hermaphroditic plants with self-incompatibility[10]. This pattern of low fruit production occurs in several tree species in the tropics[11]. Several hypotheses have been advanced for explaining the possible adaptive value and reproductive role of the 'surplus' flowers[12,13]. The large floral display with the hermaphroditic flowers in *S. nux-vomica* appears to play most of the proposed roles of the 'surplus' flowers such as attractive device to pollinators, increases pollen production and presentation time thus enhancing the male function, and provides a buffer against loss of reproductive units due to damage by aphids and sooty mould fungus. Further, the fruit in *S. nux-vomica* takes a long time of 10-11 months for complete development and requires more resources hence the low fruit - flower ratio[14].

The number of mature seeds per fruit is also low in *S. nux-vomica.* Embryological studies found ovule degeneration as a common phenomenon in this taxon[15]. Artificial pollinations in *S. nux-vomica* failed to improve the seed number, hence ovule abortion may not be attributed to lack of adequate pollination and fertilization. Stephenson and Berlin[16] stated that in some plants with several ovules per ovary, a fixed proportion of ovules will invariably abort whether or not fertilization occurs. Thus, in *Cryptanthus flava* only one of the four ovules[17], in *Madhuca indica* only one of the eight ovules[18], and in *Syzygium cumunii* only one of the 25-30 ovlules[19] develops into mature seed. *S. nux-vomica* may be having a similar tendency, and the number of 3 - 4 seeds per fruit may be specific for this taxon. Such ovule abortions are linked to plants with breeding systems whose gene pools harbour more genetic variation[20,21]. Being a long-lived tree species, *S. nux-vomica* might accumulate a greater mutation load, and therefore should favour out-crossing in order to shed the genetic lethals[21,22]. Cross-fertilization in this taxon, with mixed matings, might be facilitated by functional self-incompatibility termed as 'cryptic self-incompatibility'[23], or by the Darwin's prepotency of out-crossing pollen[24]. Such a mechanism is based on competitive discrimination against self-pollen rather than positive inhibition of self-pollen function[25]. Mixed pollen may be deposited on the stigmatic surfaces under the pollinator activity. The superior functioning of the microgametes of xeno-pollen over those resulting from self-pollen has been well established[26,27,28]. Also it is known that self-fertilization in species with mixed mating increases the number of ovules/seeds that abort[21]. The seed-ovule ratio in *S. nux-vomica* is approximately 1 : 6. Such lower S/O ratio usually occurs in out-crossing woody plant species[29]. Obviously, cross-fertilization of ovules in *S. nux-vomica* is facilitated by cryptic self-incompatibility and the cross-fertilized ovules display their dominance and develop into mature seeds.

Acknowledgement

The work was carried out in a research project sanctioned to the first author by CSIR, Government of India. Financial assistance of CSIR is duly acknowledged.

References

1. Subba Reddi C, Reddi NS. Pollen production in some anemophilous angiosperms. *Grana* 1986; **25** : 55-61.
2. Cruden RW. Pollen-ovule ratios: A conservative indication of breeding systems in flowering plants. *Evolution* 1977; **31** : 32-46.
3. Rangaswami G, Mahadevan A. Diseases of crop plants in India. New Delhi: Prentice Hall of India, 1999.
4. Ivri Y, Dafni A. The pollination ecology and *Epipactis consimilis* Don (Orchidaceae) in Israel. *New Phytol* 1977; **79** : 173-177.
5. Sivaraj N, Krishnamurthy KV. Flowering phenology in the vegetation of Shervaroys, South India. *Vegetatio* 1989; **79** : 85-88.
6. Feagri K, vander Fiji L. The principles of pollination ecology. Oxford: Pergamon Press, 1979.
7. Lloyd DG, Yates JMA. Intrasexual selection and the segregation of pollen by stigmas in hermaphroditic plants, exemplified by *Wahlenbergia albomargianata* (Canipanulaceae). *Evolution* 1982; **36** : 903-913.
8. Primack RB, Lloyd DC. Andromonoecy in the New Zealand montane shrub manuka, *Leptospermum scoparium* (Myrtaceae) *Amer J Bot* 1980; **67** : 361-368.
9. Palmer M, Travis J, Antonovics J. Seasonal pollen flow and progeny diversity in *Amianthium muscaetoxicum :* ecological potential for multiple mating in a self-incompatible hermaphroditic perennial. *Oecologia* 1988; **77** : 19-24.
10. Sutherland S, Delph L. On the importance of male fitness in plants: Patterns of fruit-set. *Ecology* 1984; **65** : 1093-1104.
11. Bawa KS, Webb CJ. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. *Amer J Bot* 1984; **71** : 736-751.
12. Holtsford TP. Non-fruiting hermaphroditic flowers of *Calochortus leichtlinii* (Liliaceae): Potential reproductive functions. *Amer J Bot* 1985; **72** : 1687-1694.
13. Ehrlen J. Why do plants produce surplus flowers? A reserve-ovary model. *Amer Nat* 1991; **138** : 918-933.
14. Sutherland S. Pattern of fruit set: What controls fruit flower ratios in plants? *Evolution* 1986; **40** : 117-128.
15. Maheswari Devi H, Lakshminarayana K. Embryological studies in Loganiaceae. *J Indian Bot Soc* 1980; **59** : 370-375.
16. Stephenson AG, Bertin RI. Male competition, female choice, and sexual selection in plants. In: Real L, editor. Pollination Biology . Orlando: Academic Press, 1983; pp. 109-149.
17. Casper BB, Wiens D. Fixed rates of random ovule abortion in *Cryptanthus flava* (Boraginaceae) and its possible relation to seed dispersal. *Ecology* 1981; **63** : 866-869.
18. Kuruvilla PK. Pollination biology, seed setting and fruit setting in *Madhuca indica* (Sapotaceae). *Indian Forester* 1989; **115** : 22-28.
19. Krishnamurthy KS, Uma Shaanker R, Ganeshaiah KN. Seed abortion in an animal dispersed species, *Syzygium cuminii* (L.) Skeels (Myrtaceae): The chemical basis. *Curr Sci* 1997; **73** : 869-873.
20. Weins M, Calvin CL, Wilson CA, Davern CI, Frank D, Seavey SR. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. *Oecologia* 1987; **71** : 501-509.
21. Ledig FT. Heterozygosity, heterosis, and fitness in outbreeding plants. In: Soule M, editor. Conservation biology. The science of scarcity and diversity. Sunderland: Sinauer, 1986; 77-104.
22. Arroyo MTK, Squeo F. Relationship between plant breeding systems and pollination. In: Kawano S, editor. Biological approaches and evolutionary trends in plants. London:Academic Press, 1990; 205-227.
23. Bateman AJ. Cryptic self-incompatibility in the wallflower: *Cheiranthus cheiri L. Heredity* 1956; **10** : 257-261.
24. Lloyd DG, Schoen DJ. Self-and cross-fertilization in plants. 1. Functional dimensions. *Int J Plant Sci* 1992; **153** : 358-369.
25. Molau M, Carlsson M, Dahlberg A, Hill O. Mating system and pollen- mediated gene flow in *Bartsia alpina. Oikos* 1989; **55** : 409-419.
26. Mulcahy DL, Curtis PS, Snow AA. Pollen competition in a natural population. In: Jones C, Little JR, editors. Handbook of,.fxperimental pollination biology. New York: Van Nostrand Reinhold Company Inc, 1983; 330-337.
27. Marshall DL, Ellstrand NC. Sexual selection in *Raphanus sativus:* Experimental data on nonrandom fertilization, maternal choice, and consequences of multiple paternity. *Amer Nat* 1986; **127** : 446-461.
28. Snow AA. Pollination dynamics in *Epilobium canum* (Onagraceae) : consequences for gametophytic selection. *Amer J Bot* 1986; **73** : 139-151
29. Wiens D. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. *Oecologia* 1984; **64** : 47-53.

Axillary shoot multiplication from nodal explants of the sweet basil *Ocimum basilicum* L.

SHANTHY SUNDARAM and SANTOSH KUMAR SINGH

Centre for Biotechnology, Nehru Science Centre, University of Allahabad, Allahabad-211002, India.

Received August 3, 2006, Revised July 28, 2007, Accepted August 2, 2007

Abstract

Sweet Basil *(Ocimum basilicum)* is an important medicinal herb and is called 'Medicine Mughal' by many. It is used to prepare herbal formulations to cure different diseases. Its mosquito repellant property can also be exploited as an alternative to synthetic repellants used presently. An efficient protocol for *in vitro* shoot multiplication of Sweet Basil has been developed. Nodal segments, from young plants, were taken as explants; shoot multiplication was induced on slightly modified Murashige and Skoog's (MS) medium supplemented with BAP (2.66 μM) and NAA (4.84 μM). Shoot proliferation could be induced using different combinations of BAP, IAA and Kinetin. Shoots were further multiplied through continued subculture of nodal segments with sprouted shoots. Micro-shoots were rooted in the basal medium supplemented with IAA (1.71 μM) and BAP (0.44 μM). Survival of *in vitro* grown plantlets 2 months after transplantation in the pots, containing equal parts of sand and top soil, was found to be 95 percent.

Key words : micropropagation, plant growth substances, multiple shoots, *Ocimum basilicum*.

सारांश

मीठी तुलसी *(आसिमम बैसीलिकम)* एक महत्वपूर्ण औषधीय शाक है।कई अन्वेषकों द्वारा इसे 'मेडिसिन मुगल' भी कहा जाता है। इसका उपयोग विभिन्न रोगों की चिकित्सा में प्रयुक्त, कई जड़ी बूटी सम्बन्धी सूत्रों में होता है। इसके मच्छर प्रतिकारक गुण को कृत्रिम प्रतिकारकों के विकल्प के रूप में प्रयुक्त किया जा सकता है। प्रयोगशाला में मीठी तुलसी के तना-संवर्धन एवं गुणन के लिये एक सक्षम मूलप्रति का विकास किया गया है। प्रयोगिक पदार्थ के रूप में अल्पविकसित पर्व संधि-युक्त खण्डों को प्रयुक्त किया गया। तना-संवर्धन प्रक्रिया को आंशिक रूप से परिवर्तित एवं BAP (2-66 μM) और NAA (4-84 μM) युक्त मुराशिगे तथा स्कूग (एम0एस0) माध्यम में उत्प्रेरित किया गया। तना-संवर्धन बी0ए0पी0, आई0ए0ए0 एवं काइनेटिन के विभिन्न संयोजनों में प्रेरित किया जा सकता है। तनों एवं पर्व संधि युक्त कोपलों के संवर्धन को सतत जारी रखा गया। सूक्ष्म विकसित तनों में जड़ों का विकास IAA (1.71 μM) एवं (0.44 μM) युक्त आधारीय माध्यम में किया गया। प्रयोगशाला में संवर्धित पौधों का रोपण, बराबर अनुपात में मिट्टी एवं बालू युक्त गमलों में किया गया एवं उनका जीवित एवं विकसित होने का प्रतिशत 95% तक पाया गया।

सांकेतिक शब्द : सूक्ष्म प्रसार, पौधीय वृद्धि पदार्थ, बहुखण्डीय तने, *आसिमम बैसीलिकम*।

Introduction

Ocimum basilicum L. (Family Labiatae) is commonly known as Sweet Basil (Meethi Tulsi). In villages of eastern Uttar Pradesh, it is called as Mamari or Bobai Tulsi. This is a herbaceous, annual plant whose height ranges from 0.1-0.6 m. Stem is glabrous with minute hair concentrated on two opposing faces of the stem; calyx pilose or pubescent (Type: Western Asia, Linnaean Herbarium 749.5, Lectotype, LINN).[1]

It is a native of tropical Asia and northeast Africa. The species basically grows on disturbed grounds, prone to flooding and also in grasslands. It is cultivated in many regions throughout North Africa, Europe and South West Asia. *Ocimum basilicum* is a prominent mosquito repellant. Plant twigs are also used in some regions of the state of Uttar Pradesh to repell mosquitoes in homes. It is also used as a general promoter for health in herbal medicine[2] and its other properties range from antistress, antimicrobial[3], hepatoprotective[4], radioprotective, anticancerous, anticonvulsant[5] and antioxidant[6] to name a few.

Material and Methods

Seeds *of Ocimum basilicum* were collected from naturally growing wild plants in parts of district Azamgarh, Uttar Pradesh. The seed grown plants were raised in the greenhouse at the Centre of Biotechnology, University of Allahabad. Their identification was confirmed by comparison with the type specimen at the Central Circle, Botanical Survey of India, Allahabad and Arogyadham, Chitrakoot. Nodal explants were taken from young and healthy plants for culture initiation.

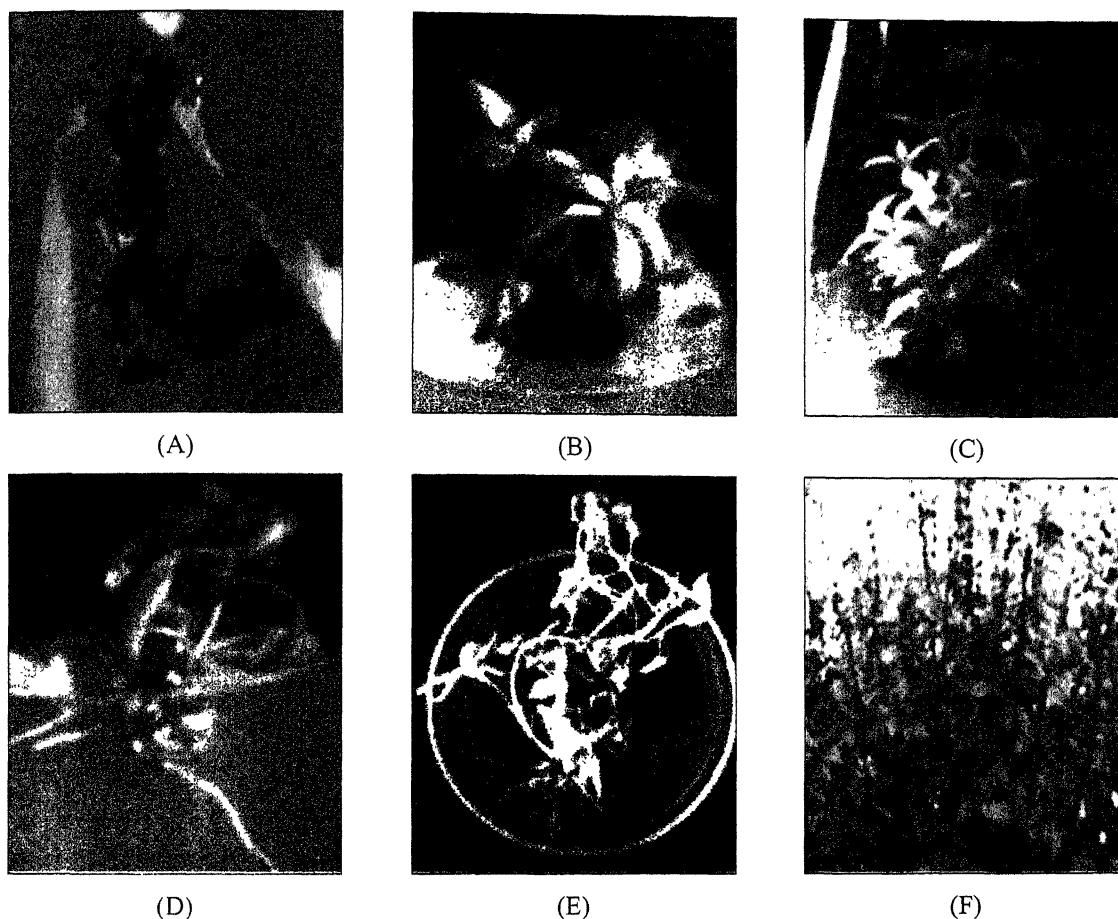



Fig. 1 - *In vitro* propagation of Sweet Basil.

(A) Origin of multiple shoots from nodes, after 3 weeks on MS medium supplemented with BAP (2.66 mM) and NAA (4.84 uM). (B) multiple shoot formation after 25 days on MS medium supplemented with 2.22 mM BAP only. (C) mass multiplication of shoots after 5 weeks (14 shoots) on the MS medium supplemented with BAP (4.44 mM) and IAA (2.86 mM). (D) development of healthy and viable roots on medium supplemented with IAA (1.71 mM) and BAP (0.44 mM) after 18 days. (E) a 6 week old plant ready to be planted in pots. The survival of such plantlets, 4 weeks after plantation, was found to be more than 74%. (F) tissue culture plants growing in the field after 6 months.

Explants were first washed in running tap water. Afterwards; the explants were placed in a beaker containing water; 3-4 drops of Tween-20 were added and the beaker was shaken lightly for 3-4 minutes, followed by thorough washing with tap water (x 4) and double distilled water (x 4). Surface disinfection was carried out with 0.1% $HgCl_2$ (w/v) for 4 minutes[7] followed by several washings with sterilized distilled water. The explants were allowed to dry in laminar hood for 20 minutes to remove surface water, and the nodal segments were then inoculated under aseptic conditions on agar solidified Murashige and Skoog's (MS)[8,9] medium with slight modifications (manganese sulphate dihydrate was used in place of manganese sulphate tetrahydrate and concentration of $Na_2EDTA.2H_2O$ was 30.5mg/l in place of 37.25 mg/1.). The medium was supplemented with usual salts and vitamins and 2.8% sucrose (w/v; Hi-Media), 100mg/litre myo-inositol (E. Merck) and 0.8% agar (w/v; Difco-Bacto, Becton Dickinson, U.S.A.). The medium was supplemented with various concentrations of BAP (6-benzylamino purine) alone and in combinations with NAA (a- naphthalene acetic acid). The pH of the medium was adjusted to 5.8 before the addition of agar and autoclaved at 121°C (1.06 kg/cm^2) for 17 minutes. The cultures were kept at 25 ± 2°C under

Table 1 - Effect of plant growth Substances on shoot multiplication from cultured nodal explants (values are means ± SE of five replicates per treatment)

Plant Growth Substances (conc, in µM)	20 Days		40 Days	
	No. of shoots	Average length of shoots (mm)	No. of shoots	Average length of shoots (mm)
1. Control	1	0.3±0.04	2	0.6±0.1
2. BAP (2.22)	4	0.5±0.03	10	16±0.6
3. BAP (4.44)	3	0.6±0.04	7	12±0.3
4. BAP (4.44)+IAA (2.86)	10	3.0±0.1	21	35±1.5
5. Kinetin (2.33)	4	1.0±0.3	7	29±1.0
6. BAP (1.33)+Kinetin (2.33)	4	1.1±0.3	8	26±0.8
7. BAP (2.22)+Kinetin (1.40)	7	1.8±0.6	15	28±1.3
8. Kinetin (4.65)+IAA (2.86)	5	0.8±0.1	11	28±0.9

Table 2 - Effect of plant growth substances on rooting of *in vitro* raised microshoots (values are means ± SE of five replicates per treatment)

Plant Growth Substances (conc. in µM)	20 Days	40 Days		
	No. of roots	Average length of roots (mm)	No. of roots	Average length of roots (mm)
1. Control	1	0.2+0.03	2	1.2+0.01
2. IAA 0.57	3	3+0.8	4	6+0.05
3. IAA 1.71	3	0.6+0.06	5	13+1.3
4. IBA 1.48	2	0.5+0.1	4	10+0.9
5. IAA 1.71+2,4-D 0.45*	2	0.7+0.11	5	12+0.4
6. IAA 1.71+BAP 0.44	4	0.8+0.1	7	20+0.8

(* 2, 4- D was not used for rooting because it induced basal callusing.)

illumination with white fluorescent tubes (50 µM m^{-2} s^{-1}) at 78% relative humidity. They were maintained under light for 14 hours followed by 10 hours dark period. Each treatment had 5 replicates and the experiments were repeated 3 times.

Sprouting of axillary buds was seen on nodal segments after 2-3 weeks of culture. These buds, with part of the growing nodal segments, were subcultured on modified medium supplemented with BAP (1.33-4.44 µM) + Kinetin (1.40-2.33 µM) or BAP (1.33-0.44 µM) + IAA (2.86 µM) for further shoot multiplication. Nodal explants (0.6-0.8 cm) from the axenic shoots were recultured on agar solidified medium containing different concentrations of BAP and IAA for inducing multiple shoots. Elongation of shoots was not observed in the shoot multiplication medium even after 3 weeks of incubation. All the shoots were transferred to media supplemented with GA_3 (0.17-3.30 µ M) for shoot elongation; the shoots attained height of 2.3±0.10 cm. In the shoots measuring 2-3 cm in length roots were induced by transferring them to MS medium supplemented with different combinations of IAA, BAP and 2, 4,-D. The roots were initiated in rooting medium as well as in basal medium. The roots produced in basal medium were thin and short.

Table 3 - Survival of plantlets under *ex vitro* conditions (values are means ± SE of five replicates repeated thrice)

No. of plantlets produced & transferred to pots	No. of surviving plants after 60 days	Survival percent (%)
30	28	93.3
39	38	97.4
32	29	90.0
41	39	95.12
40	38	95.0

Average = 95%

Eight weeks old plantlets were transferred to pots containing sterilized soil and sand (1:1), covered with polythene bags with perforations, for 10 days and the pots were kept below 25±2°C, for acclimatization. These were then transferred to green house for hardening, after removing polythene covers.

Results and Discussion

Best induction of multiple shoot formation from nodal explants occurred on medium containing BAP (2.66 µM) and NAA (4.84 µM). Later on the cultures were transferred to the medium that favored multiple shoot regeneration. Amongst different combinations of plant growth substances used, maximum shoot regeneration per explant occurred in BAP (4.44 µM) and IAA (2.86 µM) - (Figure 1 C) with about 21 shoots in 40 days. Use of BAP (2.22 µM) with Kinetin (1.40 µM) also gave multiple shoots (15 shoots per explant after 40 days). In contrast, in control cultures the number of shoots formed was only 2± 0.5; shoot induction was late and the shoots formed were less viable (survival only 25%). Further, it was found that higher concentrations of BAP inhibited the shoot multiplication rate as well as induction of shoots.

Root initiation was tried with different combinations of IAA, BAP, IBA and 2, 4-D. Best root growth was promoted by BAP (0.44 µM) used with IAA (1.71 µM, Figure 1 D) or with IAA alone (1.71 µM). During rooting of shoots, 2, 4-D caused rapid callusing at the base of stems, hence, not used further.

It was observed in the present investigations that multiple plant regeneration from nodal explants of *Ocimum basilicum* could be induced on slightly modified MS medium. Plant multiplication rate was dependent on appropriate combinations of plant growth substances (PGSs). Higher concentrations of PGSs, especially BAP was found to inhibit shoot multiplication. The current work provides preliminary information and methodology for rapid propagation of this valuable plant.

Conclusion

Ocimum basilicum is widely used in many traditional medicines prescribed under different systems of medicine. It is, therefore, important to maintain a balance between its use and conservation status. Propagation and conservation of some pharmaceutically important *Ocimum* species as *Ocimum americanum* L. syn. *O. canum* Sims, (hoary basil); *O basilicum* L. (sweet basil); *O. gratissimum* L. (shrubby basil); and *O. sanctum* L. (sacred basil) was attempted using synthetic seed technology[10]. Suspension cultures widely used for the *in vitro* production of secondary metabolites using large and small scale fermenters, proved the importance of tissue culture technology[11].

Some other workers have reported *in vitro* regeneration system for basil through primary callus[12]. In the present study, direct shoot multiplication was preferred for generating true-to-type plants than callus regeneration. This study supported the rapid multiplication of this useful medicinal plant *in vitro*. An efficient plant regeneration protocol was successfully developed for basil *(Ocimum basilicum* L.). *In vitro* mass propagation of Sweet Basil reported here may provide some help in this direction. The protocol developed is easy and reproducible through which its mass multiplication can be attempted at commercial level. It is less time taking and the survival rate of *in vitro* grown plants was also found to be more than 95%, a considerable improvement over earlier studies.

Acknowledgements

The authors wish to thank the Council of Scientific and Industrial Research, New Delhi for financial support in the form of a fellowship to Santosh K. Singh. They also thank the Director, Arogya Dham (Chitrakoot) and Botanical Survey of India (Allahabad) for identification of the species.

References

1. Paton A, Horley RM, Horley MM. *Ocimum:* An Overview of Classification and Relationships. Singapore: Overseas Publishers, 1999; 1-38.

2. Ashok DB, Vaidya. The status and scope of Indian Medicinal Plants acting on CNS. *Indian J Pharmacol* 1997; **29** : S-340-S-343.

3. Kiritikar KR, Basu BD. Indian Medicinal Plants, vol. II, Dehradun: B.P. Singh, M.P. Singh, 1975. 1931-1450.

4. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants Vol. V. New Delhi: Publications & Information Directorate 1995. 1-425.

5. Raj KJ, Richa M, Singh B. Anticonvulsant potential of Holy Basil, *Ocimum sanctum* Linn. and its cultures. *Indian J Exp Biol*, 2003; **41** : 1329-1333.

6. Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chemistry* 2003; **83** : 547-550.

7. Frans Worth RN. Biological and Phytochemical screening of plants. *J Pharm Sci* 1966; **55** : 225-276.

8. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Pl* 1962; **15** : 473-497.

9. Murashige T. Plant propagation through tissue culture, Annual Review, *Plant Physiol*, 1974; **25** : 136-166.

10. Mandal J, Pattnaik S, Chand P. Alginate encapsulation of axillary buds of *Ocimum americanum* L. (hoary basil), *O. basilicum* L. (sweet basil), *O. gratissimum* L. (shrubby basil), and *O. sanctum*, L. (sacred basil) *in Vitro. Cellular and Developmental Biology Plant*, 2000; **36 (4)** : 287-292.

11. Curtin NE. Harvesting profitable products from plant tissue culture. *Biotechnology*, 1983; **1** : 649-657.

12. Phillipen WB, Simon JE. Shoot regeneration of young leaf explants from basil *(Ocimum basilicum* L.) *in Vitro. Cellular and Developmental Biology Plant*, 2000; **36 (4)** : 250-254.

Proc. Nat. Acad. Sci. India, Sect. B, Vol. 78 Pt. I, 2008

Biomass productivity of *Acacia auriculiformis* as an important renewable energy resource

JITENDRA KUMAR

Department of Science, Sampurnanand Sanskrit University, Varanasi-221002, India.

Received February 9, 2007, Revised August 9, 2007, Accepted September 5, 2007

Abstract

Acacia auriculiformis A.Cunn. ex. Benth., a fast growing N_2-fixing tree, a native of Papua New Guinea, Australia, has come to India recently after its highly successful tree farming in Indonesia. At and around Varanasi, two age group plantations of *Acacia auriculiformis* stand as Site I & II. They have been studied for biomass production of bole, branch, phyllode and below ground parts.

The net primary production (NPP) of 22 - 27 t ha^{-1} yr^{-1}, obtained under natural environmental conditions with very little external inputs is regarded high. About 60% of the total NPP is contributed by bole component and 26% – 28% by branches followed by phyllode (5%-10%) and root (4%-6%) at the two sites. This high rate of annual biomass production can supplement fuel wood (energy), timber, biofertilizer and cattle fodder.

Key words : *Acacia auriculiformis*, biomass, energy resource, productivity.

सारांश

एकेसिया आरीकुलीफ़ारमिस (ए०कन०एक्स०बेन्थ०) एक तेजी से वृद्धि एवं नाइट्रोजन स्थिरीकरण करने वाला पौधा है। इसका जन्म स्थल पैपुआ न्यू ग्यूनीया, आस्ट्रेलिया है। इस पौधे को इण्डोनेशिया में सफलता पूर्वक खेती के बाद, हाल के वर्षों में भारत लाया गया। वाराणसी और इसके आस–पास, दो उम्र वर्ग के पौधा रोपड़ वाले वन क्षेत्र का चुनाव किया गया और इनके मुख्य तना (बोल), शाखाओं, पत्तियों (फिल्लोड) एवं जड़ों के बायोमास एवं उत्पादन दर का अध्ययन किया गया।

प्राकृतिक पर्यावरणीय स्थिति में बिना किसी अन्य प्रयासों के इनका शुद्ध प्राथमिक उत्पादन (एन0पी0पी0) 22–27 टन प्रति हेक्टेयर प्रतिवर्ष प्राप्त हुआ। दोनों स्थानों पर शुद्ध प्राथमिक उत्पादन में लगभग 60% मुख्य तनों की, 26%-28% शाखाओं की, 5%-10% पत्तियों की और 4%-6% जड़ों की भागीदारी रही। यह उच्च वार्षिक बायोमास उत्पादन दर वाले पौधे, ईधन (ऊर्जा), लकड़ी, जैव खाद और जानवरों के लिये चारा की आपूर्ति करते हैं।

सांकेतिक शब्द : *अकेसिया अरीकुलीफारमिस*, जैवमात्रा, ऊर्जा संसाधन, उत्पादकता।

Introduction

To provide an alternative energy source and to overcome the deteriorating ecological conditions such as wasteland formation, loss of green cover and associated climatic problems, the Uttar Pradesh Forest Department has selected a few tree species including *Acacia auriculiformis* for mass scale plantation in and around Varanasi. Biomass and productivity studies on these plantations are however, lacking.

Biomass study is fundamental for understanding the dynamics of ecological systems and biomass distribution. It helps in determining the distribution and flow of materials within the ecosystems[1]. Net primary production is the ultimate source of potential energy which would successively be used by human beings and other animal consumers. Compilation and evaluation of net primary productivity and related problems on global basis has been made by Odum[2], Ovington *et al.*[3] and Lieth and Whittaker[4]. In the present study, plantations of *Acacia auriculiformis* of known ages have been studied.

Material and Methods

Three-year old plantation stand of *Acacia auriculiformis* was available at Phulwaria forest range in the North West outskirts of Varanasi City which was selected as site-I. This area had a tree density of 245 trees per 0.1 hectare. A 6-year old plantation area at Saresar (Mughalsarai) forest range, was identified as site-II. Here the tree density was 230 trees per 0.1 hectare. Each of the two plantations were of more than five hectare size. The study sites were located at 25° 18′ North latitude and 83°1′ East longitude

in the middle Gangetic plain at approximately 79.1 meters above the sea level. The climate of the area has been tropical and all the elements of climate viz; temperature, rainfall and relative humidity show well marked variation in rainy, winter and summer seasons. Average annual rainfall of the area has been 1100 mm. The soil of the study sites is sandy loam and more or less neutral in pH. The area was divided into a sample and a buffer zone[3]. In the sample area, growth and climatic measurements were performed. The felling of trees, digging of soil pits and soil core for fine root estimation were carried out in the sample area. For regular growth studies in each area, a sample zone of 0.2 ha was marked.

Eighteen trees representing seven diameter classes were harvested in December 1995 from Phulwaria and Saresar forest range. Tree height and girth were measured for the analysis. Girth (gbh) of the main stem was measured with the help of a measuring tape at 1.3 m height from the ground. The harvested trees were separated into different components as phyllodes which function as leaves, branches of each of the felled trees which were separated carefully and weighed, bole which was cut into one meter segments and weighed, major roots of harvested trees, which were excavated and weighed.

One kilogram fresh sample of each of the different components was collected in three replicates which was oven dried and weighed. The dry weight data have been recorded in table-1. Fresh weights measured in the field were converted into dry weights.

Allometric relationship was developed using dry weight and tree diameter at breast height (dbh) for each component separately as done earlier by Ovington and Madgwick,[6], Attiwill[7], Whittaker and Woodwell[8], Whittaker *et al.*[9]. by the following formula :

$$\log_{10} Y = a + b \log_{10} X$$

where,

Table 1 - Component dry weight (kg per tree) of the harvested *Acacia auriculiformis* trees.

S.N.	dbh(cm)	Bole	Branch	Foliage	Root	Total
1.	1.19	0.390	0.029	0.087	0.220	0.728
2.	1.61	0.562	0.056	0.159	0.346	1.124
3.	2.25	1.401	0.131	0.395	0.706	2.634
4.	2.64	1.767	0.259	0.470	0.745	3.242
5.	3.12	2.344	0.298	0.682	0.937	4.262
6.	3.38	2.816	0.273	0.897	0.797	4.984
7.	3.92	3.407	0.554	0.981	0.831	5.775
8.	4.20	3.694	0.692	1.000	1.026	6.414
9.	4.84	4.697	0.858	1.295	1.247	8.098
10.	5.19	5.335	1.124	1.524	1.543	9.526
11.	5.80	5.461	1.191	1.588	1.688	9.929
12.	5.99	6.681	1.758	1.992	1.289	11.721
13.	7.49	13.696	4.157	3.668	2.934	24.457
14.	8.16	15.742	4.142	4.418	3.314	27.618
15.	9.98	18.238	5.862	4.885	3.582	32.568
16.	12.34	43.478	12.368	10.119	8.995	74.962
17.	14.19	50.510	14.076	9.938	8.280	82.804
18.	15.42	65.796	20.245	16.308	10.122	112.472

Table 2 - Logarithmic regression relating biomass (dry weight in g) of bole, branch, phyllode and root as dependent variable with the function of tree DBH (in cm) as independent variable of the harvested sample trees.

S.N.	Components	Regression equations
1.	Bole	Log(Y) = 2.370455 + 1.983626 log (X)
		r = 0.9937 (P<0.01), E = 0.068367
2.	Branch	Log (Y) = 1.221129 + 2.580178 log (X)
		r = 0.99635 (P<0.01), E = 0.06773
3.	Phyllode	Log (Y) = 2.088389 + 1.621671 log (X)
		r = 0.96659 (P<0.01), E = 0.1526
4.	Root	Log (Y) =2.390018 + 1.1999426 log (X)
		r = 0.938124 (P<0.01), E = 0.257123

Y= Dry weight of component; X = Diameter at breast height (cm)

r= Coefficient of correlation ; E = Estimate of relative error

'Y' is the dry weight of components; 'X' is the diameter at breast height over the bark, 'a' is the intercept constant and 'b' is the slope constant.

The relative tightness of regression was effectively expressed estimating relative error 'E'[8,9]. E is the antilog of the standard error of the logarithmic of the Y-values and reflect the error in predicting logarithm of value and is more useful predictor than the least square standard error of estimate[10].

The girth was measured for each tree at the two sites, diameter of each tree at breast height (dbh) was calculated from these values. The component biomass values of the plantations were obtained by applying mean dbh of the each diameter class to the logarithmic regression equations developed for each component (Table 2), and multiplying it by the number of trees in the diameter class. The component biomass value of each diameter class of the plantation stand was added and converted to stand biomass.

The dbh of the trees was measured in the month of December 1996 and 1997 for two successive years. With the help of calculated mean dbh values and regression models, component biomass values were predicted and by summing the component biomass values, the total biomass of the plantation area was obtained for both the study sites. Thus the annual increment in the component biomass of trees was calculated by dbh increments.

Net annual primary production was calculated by adding the increment of biomass between two successive years over a unit land area.

Results and Discussion

The diameter of trees at breast height (dbh) ranged from 1.19 cm to 15.45 cm, and total dry weight from 0.728 kg to 112.472 kg. The highest biomass was contributed by the bole component (Table 1). The bole biomass increased with tree dbh as also found by Roy *et al.*[11]. The dry weight of the different components increased with increasing diameter classes. The dry weight contribution of bole, branch and phyllode components to the total biomass were 53.60%-58.49%, 4.09%-17.99% and 12%-14.49%, respectively. The root biomass percentage contribution was 3%-9% and it decreased with increasing dbh classes.

The mean component biomass of both the sites (I and II) have been calculated for three successive years 1995, 1996, and 1997. At site I, the total biomass ranged from 24.72 t ha^{-1} in 1995 to 75.26 t ha^{-1} in 1997. The component biomass increased with age (Table 3).The bole component biomass contributed the highest percentage to the total biomass, followed by branch, root and the phyllode[12]. The above ground biomass represents 85% to 89% of the total biomass. The above ground biomass value increased with increase in mean dbh classes as also found by Dhana *et al.*[13] in *Leucaena leucocephala*. The fruit formation was missing during harvesting period of the trees at the study sites.

Table 3 - Estimates of biomass in the *A.auriculiformis* plantation stand. Percent values are given in parenthesis.

Component	Biomass (t ha^{-1})		
	1995	1996	1997
Site-I			
Bole	13.97	28.36	44.68
	(56.51)*	(58.77)	(59.37)
Branch	3.28	7.22	12.04
	(13.27)	(14.96)	(16.00)
Phyllode	3.93	6.86	10.29
	(15.90)	(14.21)	(13.67)
Root	3.54	5.82	8.25
	(14.32)	(12.06)	(10.96)
Total	24.72	48.26	75.26
Site-II			
Bole	58.64	73.63	86.18
	(59.77)	(59.86)	(59.76)
Branch	16.35	23.44	29.00
	(16.67)	(19.06)	(20.11)
Phyllode	13.97	15.28	17.35
	(14.24)	(12.42)	(12.03)
Root	09.14	10.65	11.69
	(11.36)	(08.66)	(08.10)
Total	98.10	123.00	144.22

*Values given in the parenthesis are percent biomass contribution of different component to the total biomass.

The stand biomass at site II ranged from 98.10 t ha^{-1} in 1995 to 144.22 t ha^{-1} in 1997. Here also, the bole component shared highest percentage of the total biomass, followed by branch, phyllode and the root. The above ground biomass percentage contribution was 90%-92% of the total biomass.

Seasonal variation and distribution of fine root biomass in the plantation areas of *Acacia auriculiformis* were estimated at both the sites for the period of one year (December 1995 to December 1996). It is observed that the fine root biomass is nearly equal in the winter and rainy seasons at both the sites. Maximum fine root biomass of 177 g m^{-2} at site I and 187 g m^{-2} at site II was found in the rainy season in the top 10 cm soil depth and it decreased gradually with increasing soil depth. Lowest fine root biomass of 38 g m^{-2} at site I and 42 g m^{-2} at site II, was observed during summer season at 10-20 cm depth. Higher fine root biomass was observed during summer and rainy seasons at 0-10 cm depth, whereas during winter seasons the 20-30 cm depth showed higher biomass values at both the sites.

The total above ground and below ground net annual primary production of the plantation area was estimated for the years 1995-1996 and 1996-1997, at the two study sites. The net primary production of the plantation area increased with age. At site I, the biomass production value was 23.54 t ha^{-1} yr^{-1} in 1995-96,

Table 4 - Component wise and total net primary production (t ha^{-1} $year^{-1}$) of *Acacia auriculiformis* plantation stand.

Year	Bole	Branch	Phyllode*	Root**	Total
Site-I					
1995-1996	14.31	3.94	2.93	2.28	23.54
1996-1997	16.32	4.82	3.43	2.43	27.00
Site-II					
1995-1996	14.99	7.09	1.31	1.51	24.90
1996-1997	12.55	5.56	2.07	1.04	21.22

* Litter fall values are not included . ** Fine root values are not included.

and 27 t ha^{-1} yr^{-1} in 1996-97 (Table 4). Bole component contributed 59% to 60% of net primary production of the area, followed by branch (16% to 18%), phyllode (12% to 13%) and root (9% to 10%). Thus the bole wood biomass contribution remained highest at all the ages. Faiz mohsin *et al.*[14] have also found this trend in their studies of *Eucalyptus* hybrid. The above ground net primary production contributed about 89.98% to the total net primary production. The total net annual primary production at site II ranged from 24.90 t ha^{-1} yr^{-1} in 1995-96 to 21.22 t ha^{-1} yr^{-1} in 1996-97. About 60% of the total net primary production was contributed by bole component followed by branch (26% to 28%), phyllode (5.26% to 9.76%) and root (4% to 6%). The 93% of the total net primary production was contributed by the above ground net primary production.

The total standing biomass per tree, and the girth are found to increase rapidly during the observation period from 1995 to 1997, indicating that in young age a very high growth rate of *Acacia auriculiformis* is possible in the tropical monsoon climatic conditions of Varanasi. Kietvuttinon[15] has found that the yield per hectare was highest in plantations having the highest plant density.

The biomass values reported for other kinds of natural plantations as studied by other Indian workers ranged between 35 t ha^{-1} to 270 t ha^{-1} [16,17,18,19]. The estimated total biomass range in the present study is closely comparable to the range of 40 t ha^{-1} to 140 t ha^{-1} biomass values measured by Jung [20],Ogawa *et al.*[21] and Vyas *et al.*[22] for four sub-tropical dry forests. Murphy and Lugo[23] also measured total biomass range of 78 t ha^{-1} to 320 t ha^{-1} for a variety of dry forests and 269 t ha^{-1} to 1186 t ha^{-1} for wet tropical forests.

The ratios of contribution of component biomass to the total biomass are comparable to the results obtained by Rana *et al.*[24] for sal forests in which component biomass ranges were : bole 55%, root 20%, branch 14% and foliage 10%. Tsai[25] has also reported almost similar ratio and range of biomass apportionment in *Acacia magnum.* During 1995-97 the phyllode biomass contribution decreased from 15% to 13%, at site I and 14% to 12% at site II, to the total biomass. The percentage contribution of phyllode decreased with the increase in tree age. The percentage contribution of phyllode at mature stage is very much nearer to the value calculated by Rodin and Bazilevich[26], Sharma[27] in temperate forest and Singh RP[28] in tropical forests. The present above ground biomass range covers the 90 t ha^{-1} -93t ha^{-1} above ground biomass values estimated by Sinha[29] for *Leucaena leucocephala* and Singh[30] for Sal dominated mixed dry deciduous forest.

Murphy and Lugo[31] have reported 28 t ha^{-1} - 266 t ha^{-1} for a variety of tropical forests. The biomass allocation in different components of Sal, Pine and Oak forests was maximum in bole, and minimum in leaves. However, in all the dry forests, the branches contributed maximum to the total above ground biomass of 3.28 t ha^{-1}-12.04 t ha^{-1} at site I and 16.35 t ha^{-1} at site II. The percentage contribution of branch component biomass to the total biomass increased from 13.27% to 16% during 1995 to 1997 at site I and 16.67% to 20.11% at site II. The percentage biomass contribution of root to the total biomass decreased with the increasing age, but the below ground biomass values increased with maturity at both the study sites which ranged from 3.54t ha^{-1} to 8.25t ha^{-1} at site I and 9.14t ha^{-1} to 11.69t ha^{-1} at site II. This is comparable to the work of Sharma *et al.*[18]

They reported it from 9.6 t ha^{-1} to 36 t ha^{-1} for the root component in Alder forests in Darjeeling Hills. The biomass distribution of different components and its percentage contribution to the total biomass are given in table -3.

The determination of fine root biomass of *Acacia auriculiformis* plantation in the present study ranged from 1760 kg-3820 kg per hectare. This is well within the range of fine root biomass of 400-6000 kg ha^{-1} as reported by Srivastava *et al.*[19]. The maximum fine root biomass of 3570 kg ha^{-1} at site I and 3820 kg ha^{-1} at site II have been recorded. The maximum biomass distribution of 1770 kg ha^{-1} to 1870 kg ha^{-1} at 0-10 cm soil depth and minimum biomass distribution of 680-790 kg ha^{-1} at 20-30 cm depth at both the study sites I and II, respectively were recorded during rainly season. It perfectly confirms that the fine roots are aggregated near the soil surface[32]. Kimmins *et al.*[33] and Persson[34] found that the majority of fine roots are confined to top 30 cm depth of the soil profile. The maximum fine root biomass contribution of 60% to 85% of the total fine root biomass occurs in upper 30cm of the soil depth. Santantanio *et al.*[35] also reported that the larger proportion of fine root biomass was centered near 20-30 cm soil profile.

The net primary production in the plantation stand of *Acacia auriculiformis* was 23.54 t ha^{-1} yr^{-1} in 1995-1996 and 27 t ha^{-1} yr^{-1} in 1996-1997 at site I. Whereas at site II, the values were 24.90 t ha^{-1} yr^{-1} in 1995-1996 and 21.22t ha^{-1} yr^{-1} in 1996-1997. In the present study, the recorded net primary production is higher than 15 t ha^{-1} yr^{-1} reported by Singh and Mishra[36] in an open tropical dry deciduous forest in India. However, a high production rate of 36 t ha^{-1} yr^{-1} has been reported by Lieth[37] for tropical rain forest. The calculated values are covering the range of 13.6 t ha^{-1} yr^{-1} to 28.6 t ha^{-1} yr^{-1} as reported by Bullock[38] and Bernhard- Reversalt *et al.*[39] for a variety of different rain forests. The NPP values of the present study are also comparable to the upper range of 13.28 t ha^{-1} yr^{-1} as reported by Murphy and Lugo[23] for wet tropical forests.

The component percentage of net primary production to the total net primary production has also been studied for both the plantation areas (Table-4). Bole account for 60%, branch 16% followed by phyllode 12% and root 9% at site I, whereas at site II bole 59%-60%, branch 26%-28%, phyllode 5%-9% and root 4%-6% for two successive years. The biomass allocation studies in the different components of *Shorea robusta* has also made by Rana *et al.*[24] They found that bole contributed 27%-33%, branch 7.8%-17%, foliage 30%-34% and roots 16%-18% in central Himalayan forests.

Lastly, it is concluded that *Acacia auriculiformis* is a good answer to the ever increasing need of mankind for a high fuel wood biomass,phyllode, fodder and much needed purification of urban atmosphere. Hence, the species *Acacia auriculiformis* is in a better acceptable position than most others for higher green coverage, revegetation and afforestation programme of wasteland in Gangetic belt of India.

Acknowledgement

The author thanks Prof. R.S. Ambash and Dr. M.P. Singh for their critical suggestions and to the authorities of Sampurnanand Sanskrit University for facilities.

References

1. Anderson A. Productivity of forest ecosystem. In: Durigread. P. editor. Paris: UNESCO 1971. 281-288.
2. Odum EP. Organic production and turnover in old field succession. *Ecol* 1960; **41** : 34-49.
3. Ovington JD, Madgwick HAI. Distribution of organic matter and plant nutrients in a plantation of scots pine. *For Sci* 1959; **5** : 344-355.
4. Lieth H, Whittaker RH. Modelling the Primary Productivity of the world. In: Leith H, Whittaker RH. editors. Primary Production of the Biosphere. New York: Springer-Verlag 1975; 237-262.
5. Newbould PJ. Methods for estimating the primary production of forest. I BP Hand book No. 2 Oxford: Blackwell Scientific Publications, 1967.
6. Ovington JD, Madhwick HAI. The growth and composition of natural stands of birch II. The uptake of mineral nutrients. *Plant Soil* 1959; **10** : 389-400.
7. Attiwill PM. A method for estimating crown weight in *Eucalyptus* and some implication of relationship between crown weight and stem diameter. *Ecology* 1966; **47** : 795-804.
8. Whittaker RH, Woodwell GM. Dimension and production relations of trees and shrubs in the Brookhaen forest. *J Ecol* 1968; **57** : 155-174.
9. Whittaker RH , Marks PL. Methods of assessing terrestrial productivity. In: Lieth H, Whittaker R H editors. Primary

productivity of the Biosphere, Berlin Heidelberg, New York: Springer- Verlag 1975; 55-118.

10. Sprugel DG. Dynamic structure of wave generated *Abies balsamea* forests in the north eastern United States. *J Ecol* 1976; **64** : 889-912.

11. Roy MM, Pathak PS, Rai AK, Kushwaha Deepak. Tree Growth and biomass production in *Melia azedarach* on farm boundaries in a semi-arid region. *The Indian Forester* 2006; **1(132)** : 105-110.

12. Kumar J. Productivity of *Acacia auriculiformis* plantation in the Gangetic belt. *Environmental biology and Conservation* 2006; **II** : 29-35.

13. Dhana RS, Dhillon GPS, Sharma SC. Timber volume biomass tables for *Leucaena leucocephala* (Lam.) De. Wit. from Punjab, India. *The Indian Forester* 2006; **8 (132)**: 931-940.

14. Faiz Mohsin, Singh RP, Rajput DK. Biomass of *Eucalyptus* hybrid plantation under agroforestry system. *The Indian Forester* 2003; **8 (129)** : 1037-1040.

15. Kietvuttinon B. Yield- density effect *of Acacia auriculiformis.* A. Cunn. ex.Benth. plantation. MSc. Thesis. Kasetsart University, Thailand, 1985.

16. Desh Bandhu . A study of productive structure of tropical dry deciduous forest at Varanasi. Ph.D. Thesis. Banaras Hindu University, Varanasi, 1971.

17. Farooqi Q. Organic and mineral structure and productivity of plantations of Sal and Teak. Ph.D. Thesis. Banaras Hindu University, Varanasi, 1972.

18. Sharma DC, Taneja PL, Bist APS. Biomass, productivity and nutrient cycling in a *Dalbergia sisoo* platation. *Indian For* 1988; **114 (5)** : 261-268.

19. Srivastava AK, Ambasht RS. Biomass production, decomposition and nitrogen release from root nodules in *Casuarina equisetifolia* plantation in Sonebhadra, India. *Jour Appl Ecology* 1995; **32** : 121-127.

20. Jung G. Cycles biogio chemique dane un ecosystem sihe *Acacia albida* (Dol.) Sol ferrugine ux tropical peu lessive peu lenive (Dior.). *Oecologia Plantarum* 1969; **4** : 195-210.

21. Ogawa H, Yoda K, Ogina K , Kira T. Comparative ecological studies on three main types of forest vegetation in Thailand. II. Plant Biomass, *Nature and life in South East Asia* 1965; **4** : 49-80.

22. Vyas LN, Garg RK, Vyas ML. Stand structure and above ground biomass in dry deciduous forest of Aravalli hills at Udaipur (Rajasthan), India. *Biologia (Bratistara)* 1977; **32** : 265-270.

23. Murphy PG, Lugo AE. Structure and Biomass of a subtropical dry forest in Puerto Rico. *Biotropica* 1986; **18**: 89-96.

24. Rana BS, Singh SP, Singh RP. Biomass and productivity of central Himalayan (India) Sal *(Shorea robusta)* forest. *Trap Ecol* 1988; **29 (2)** : 1-7.

25. Tsai LM. Studies on *Acacia magnum* in kemasul forest. Malaysia. Biomass and productivity *J Trop Ecol* 1988; **4(3)** : 293-302.

26. Rodin LE, Bazilevich NI. Production and Mineral cycling in Terrestrial vegetation (English Translation) Fogg GE, editor. London: Oliver and Boyd, 1967. 1-288.

27. Sharma E. Primary Production, Nutrient dynamics and decomposition in Actinorhizal *Alnus nepalensis* Plantations in the Eastern Himalaya (India). Ph.D. Thesis, Banaras Hindu University, Varanasi, 1985.

28. Singh RP. A Study of Primary productivity and Nutrient cycling in Chakia forest Varanasi. Ph.D. Thesis, Banaras Hindu University, Varanasi, 1974.

29. Sinha M. Primary Production, nutrient dynamics and decomposition in a plantation stand of *Leucaena leucocephala* (Lam) de. Wit. Ph.D. Thesis, Banaras Hindu University, Varanasi, 1991.

30. Singh R P. Primary Production and Energy Dynamics of Tropical Dry Deciduous Forest in Chandraprabha Region, Varanasi. Ph.D. Thesis, Banaras Hindu University, Varanasi, 1979.

31. Murphy PG, Lugo AE. Ecology of Tropical dry forest. *Annual Review of Ecology and Systematics* 1986; **17** : 67-88.

32. Jordan CF. Productivity of a tropical forest and its relation to a world pattern of energy storage. *J Ecol* 1971; **59** : 127-142.

33. Kimmins JP, Hawkes BC. Distribution and Chemistry of fine roots in British Columbia: implication of management. *Can J For Res* 1978; **8** : 265-279.

34. Persson H. The distribution and productivity of fine roots in boreal forests. *Plant Soil* 1983; **71** : 87-101.

35. Santantanio D, Hermann RK, Overton WS. Root biomass studies in forest ecosystems. *Pedobiologia* 1977; **17** : 1-31.

36. Singh KP, Mishra R. Structure and function of natural modified and silvicultural ecosystem in Eastern Uttar Pradesh. Final Technical Report MAB Research Project. Banaras Hindu University, Varanasi, 1979.

37. Lieth H. Primary production of the major vegetation units of the world. In: Lieth H, Whittaker R H, Editors. Primary productivity of the Biosphere, Berlin, Heidelberg, New York: Springer-Verlag 1975; 203-215.

38. Bullock JA. In: Reichle DE editor. Dynamic properties of forest Ecosystems. Cambridge: Cambridge University Press, 1981.

39. Bernhard - Reversal F, Huttel C, Lemee G. Structure and function of evergreen rain forest ecosystem of the Ivory Coast. In: Tropical forest ecosystems. UNESCO, *Natural Resource Research* 1978; **XIV** : 557-574.

40. Satoo T. A Synthesis of studies by the harvest method: Primary production relations in the temperate deciduous forest of Japan. In: Reichle DE edcitor. Analysis of Temperate Forest Ecosystems, New York: Springer-Verlag, 1970; 55-72.

Proc. Nat. Acad. Sci. India, Sect. B, Vol. 78 Pt. I, 2008

Studies on the role of surface micromycetes in causing post-harvest fruit rot of apple cv. red delicious

YASHPAUL SINGH and GEETA SUMBALI

Mycology and Plant Pathology Laboratory, Department of Botany, University of Jammu, Jammu-180006, India.

Received February 22, 2007, Accepted April 10, 2007

Abstract

Survey of microfungi existing on the fruit surface of apple cv. Red Delicious was carried out during post-harvest phase. Relative importance of surface micromycetes in causing spoilage of apple fruits was also assessed. During the survey, a total of 32 microfungi belonging to 20 genera were isolated by surface washing technique. Artificial inoculations with the recovered surface mycopropagules were performed in apple fruits and most of them were able to cause fruit rot with different intensity. However, *Aspergillus fumigatus* Fresenius. *A. terreus* Than. *Memnoniella levispora* Subram. *Scopulariopsis brumtii* Salvanet-Duval. *Trichurus spiralis* Hasselbring and *Verticillium lecani* Zimmerm, althoug'' present on the fruit surface, were not able to cause rot, indicating iack of specific macerating enzymes necessary for post-harvest pathogenesis.

Key words : Apple, surface microfungi, red delicious, pathogens.

सारांश

फ़सल कटने के बाद लाल स्वादिष्ट सेब की सतह पर मौजूद कक्की का सर्वेक्षण किया गया। सेब की सतह पर मौजूद विभिन्न माइक्रोमाइसिटीज़ द्वारा फलों के सडने का भी मूल्यांकन किया गया। इस सर्वेक्षण की प्रक्रिया में फलों की सतह पर 32 कक्की, जो 20 जेनेरा के थे, प्राप्त किये गये। फलों की सतह से प्राप्त किये गये विभिन्न माइक्रोप्रोपेग्यूलों का सेबों पर कृत्रिम टीकाकरण विभिन्न तीव्रता से फलों की सड़ान उत्पन्न करने में सक्षम होता है। परन्तु *ऐसपरजिलस फ्यूमीगेट्स* (फ्रेसेनियस), *ए० टेरस* (थान) *मेम्नोनिला लेवीस्पोरा* (सुब्रम), *स्कोप्यूलेरिआप्सिस ब्रम्टी* (सैल्वानेट–डुवाल), *टाइक्यूरस स्पाइरैलिस* (हैसेलब्रिंग) तथा *वर्टीसिलियम लिकैनाई* (जिर्मेम) फलों की सतह पर मौजूद होते हुये भी सड़ान नहीं पैदा कर सके, जो इस सत्य को उजागर करता है कि उनमें फल को गलाने वाला किण्वक अनुपस्थित था।

सांकेतिक शब्द : सेब, सतही सूक्ष्म कवक, लाल स्वादिष्ट, रोगाणु।

Introduction

Apple *(Malus pumila* Mill.) cv. Red Delicious, which belongs to the family Rosaceae, occupies the most important position among the various fruits of temperate region. It is known for the delicious fruits, which are rich in vitamins, carbohydrates, organic acids, calcium, phosphorus and iron. All these constituents make these fruits vulnerable to post-harvest attack by a variety of microfungi to which they are resistant during the period of their development on the plant[1]. Most of the fruit rot fungi are opportunists that may invade the fruit tissue directly or more usually through wounds attained during harvesting and poor handling practices[2]. A few researchers have monitored the fungal spectra of fruit surface and their role in causing storage rot[3]. However, very little information is available regarding the fungal population of apple fruit surface and their relative efficacy in causing post-harvest spoilage[4]. With this objective in mind, mycopropagules present on the surface of apple fruits cv. Red Delicious during marketing period were obtained in culture and their probable role in causing post-harvest fruit spoilage was evaluated by artificial inoculation.

Material and Methods

Isolation of surface micromycetes: Fungal species associated with fruit surface of harvested apples cv. Red Delicious were isolated and determined by using washing technique[5]. In this method, 25g of fruit peel was taken in an Erlenmeyer flask (250 ml containing 50 ml sterilized distilled water) and subjected to horizontal shaking for 30 minutes on a rotatary shaker. The liquid was then centrifuged at 3000 rpm for 15 minutes and the residue thus obtained was mixed with 10 ml sterilized distilled water and shaken vigorously to obtain a homogenous suspension. This suspension was poured in sterilized petriplates at the rate of 2 ml/plate. Czapek's Dox Agar medium (CDA) modified by adding

Table 1 - Percentage occurrence of fungal species associated with fruit surface of apple (cv. Red Delicious) during marketing (August, 2004 to March, 2005)

Mycoflora isolated	Sampling Months							
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Acremonium strictum W. Gams	—	—	—	—	—	1.96	0.68	1.43
Alternaria alternata (Fr.) Keisslar	3.45	1.49	7.25	14.62	13.97	14.72	8.90	2.87
Aspergillus flavus Link ex. Fries	17.24	25.37	15.38	7.01	4.24	10.78	5.48	0.72
Aspergillus niger Van Tieghem	17.24	1.49	11.10	7.75	9.09	3.92	6.84	13.66
Aspergillus ochraceus Wilhelm	—	—	—	—	—	—	1.36	0.81
Aspergillus niveus Blochwn	1.72	1.70	3.71	3.78	4.94	0.98	0.68	2.15
Aspergillus terreus Thom.	1.72	2.00	6.82	11.06	5.29	10.19	4.26	2.00
Aspergillus fumigatus Fresenius	—	18.35	6.41	2.86	—	—	3.42	—
Aspergillus recurvatus Thom.	—	2.97	3.22	7.14	0.85	0.98	—	—
Aspergillus sydowii Thom. and Church	—	—	—	—	2.41	1.96	—	—
Botrytis cinerea Pers. ex. Fries	2.60	4.00	3.28	3.12	2.90	6.00	2.12	6.01
Chaetomium globosum Kunze ex. Fries	—	3.47	—	1.00	—	—	—	—
Cladosporium oxysporum Berkand Cult.	—	—	—	6.89	—	2.12	—	7.65
Colletrotrichum gloeosporioides (Penz.) Sacc.	—	—	2.20	—	5.00	8.00	8.12	—
Emericella nidulans (Eidam) Vuill.	1.72	1.07	2.84	1.72	0.80	2.50	10.75	8.81
Fusarium solani (Mart.) Sacc.	1.70	3.40	2.05	2.44	0.64	1.96	4.10	2.15
Fusarium sporotrichioides Sherb.	—	—	—	—	2.64	1.96	—	—
Fusarium verticillioides (Sacc.) Niren-berg	3.41	2.00	2.10	1.72	0.64	2.00	—	1.43
Gliocladium roseum Bain.	—	—	3.84	1.72	3.00	3.00	4.00	0.75
Lasiodiplodia theobromae (Pat) Griff and Manble	2.04	6.14	6.80	1.72	10.58	2.94	2.40	1.67
Memnoniella levispora Subram.	—	—	—	—	1.98	2.00	—	—
Penicillium expansum Link ex. Grey	18.91	10.00	10.90	11.72	12.58	10.94	30.24	40.40
Penicillium chrysogenum Thom.	6.89	5.46	2.34	1.00	3.24	2.00	—	—
Penicillium citrinum Thom.	8.32	—	—	—	0.64	0.98	—	—
Penicillium purpurogenum Stoll	5.12	1.70	2.84	2.58	2.04	—	—	—
Paecilomyces variotii Bainier	—	—	—	—	—	—	—	4.31
Rhizopus stolonifer (Ehrenb) Lind	2.10	1.04	3.13	3.45	4.00	2.00	2.73	2.15
Scopulariopsis brumtii Salvanet-Duval	—	—	—	—	2.94	2.15	—	—
Trichoderma koningii Rifai	1.72	2.98	1.28	2.58	1.29	—	—	—
Trichurus spiralis Hasselbring	—	—	—	—	—	2.00	—	—
Trichothecium roseum (Pers.) Link ex. Fries	4.06	3.20	2.10	5.17	2.41	1.08	3.92	1.03
Verticillium lecanii (A. Zimmerm) Viegas	—	2.07	1.00	0.95	3.89	0.88	—	—

---, Not detected

Table 2 - Extent of apple (cv. Red Delicious) fruit rot caused by some surface fungal organisms isolated during post-harvest period.

Fungal species	Percentage rot	Reaction index
Acremonium strictum W. Gams	5.4 ± 0.6*	Tolerant
Aspergillus fumigatus Fresenius	No rot	Resistant
Aspergillus recurvatus Thom.	11.0±1.3	Tolerant
Aspergillus terreus Thom.	No rot	Resistant
Chaetomium globosum Kunze ex. Fries	5.6 ± 0.6	Tolerant
Cladosporium oxysporum Berk and Cult	2.6 ± 0.7	Tolerant
Emericella nidulans (Eidam) Vuill.	8.1±0.5	Tolerant
Fusarium sporotrichioides Sherb	24.0 ±1.0	Moderately susceptible
Memnoniella levispora Subram.	No rot	Resistant
Paecilomyces variotii Bainier	8.8 ± 4.0	Tolerant
Penicillium citrinum Thom.	33.8±1.6	Susceptible
Penicillium purpurogenum Stoll	31.0 ± 2.4	Susceptible
Scopulariopsis brumtii Salvanet-Duval	No rot	Resistant
Trichoderma koningii Rifai	100.00	Highly susceptible
Trichurus spiralis Hasselbring	No rot	Resistant
Verticillium lecanii (A. Zimmerm.) Viegas	No rot	Resistant

* Each value represents the mean ± SD, n = 3

streptomycin sulphate (0.06 g/1) and Rose Bengal (0.20 g/1) was used and five replicates were maintained for each sample. The plates were incubated at 28±2°C for 7 days and the resulting fungal colonies subcultured on potato dextrose agar (PDA) medium and identified. Percentage abundance of each fungal isolate was calculated as follows:

$$\text{Abundance (\%)} = \frac{\text{Number of isolates of a particular fungal species}}{\text{Total number of fungi isolated}} \times 100$$

Pathogenic potential of surface microfungi: Tests were conducted to check the pathogenic ability of the recovered surface microfungi and assess their relative importance in the spoilage of apples. For these tests, apples cv. Red Delicious of similar size and approximate maturity were surface sterilized with 70% alcohol and then inoculated with the test fungus by the pinprick method[6]. Inoculum used in each case was 5 days old culture grown on PDA at 28±2°C. After inoculation, the fruits were placed in sterilized glass chamber maintained at 28±2°C and 100% relative humidity. Extent of spoilage in each case was evaluated after 10 days of incubation[7] as follows:

$$\text{Rot (\%)} = \frac{W-w}{W} \times 100$$

Where, W = weight of the fruit before inoculation

w = weight of the fruit after removal of rotten tissue.

Results and Discussion

Microfungi of apple fruit surface cv. Red Delicious: Results depicted in Table 1 show that 32 fungal species were associated with the fruit surface of healthy apples cv. Red Delicious during marketing period. Members of the genus *Aspergillus* were predominant followed by *Penicillium* and *Fusarium* species. Other genera isolated as components of the micromycetes included

Acremonium, Alternaria, Botrytis, Chaetomium, Cladosporium, Colletotrichum, Emericella, Gliocladium, Lasiodiplodia, Memnoniella, Paecilomyces, Rhizopus, Trichoderma, Trichurus, Trichothecium, Scopulariopsis and *Verticillium*. These genera may have reached the fruit surface directly from the orchard during picking and packing operations or they may have been carried along with the packing leaves, straw and baskets or may have originated within the enclosure of the fruit shop.[8]

Data presented in Table 1 show that *Alternaria alternata* (Fr.) Keisslav, *Aspergillus flavus* Link ex. Fries, *A. niger* Van Tieghem, *A. niveus* Blochwitz, *A. terreus* Thom., *Botrytis cinerea* Pers. ex. Fries, *Emericella nidulans* (Eidam) Vuill, *Fusarium solani* (Mart.) Sacc., *Lasiodiplodia theobromae* (Pat) Griff and Mauble, *Penicillium expansum* Link ex. Grey, *Rhizopus stolonifer* (Ehrenb.) Lind, and *Trichothecium roseum* (Pers.) Link ex. Grey were the most frequently isolated components of the surface micro fungi appearing during the entire period of transit. Interestingly, most of these fungal species were found to cause spoilage of apple cv. Red Delicious during marketing[9], indicating thereby, that propagules of these fungi possess the potential to cause fruit rot. It is likely that during transportation and marketing, injuries of various kinds are caused, which facilitate the entry of these surface mycopropagules resulting in rot development. In addition, as the fruits continue to respire even after harvesting, the resultant heat accelerates respiration and aging, which in turn makes the fruit susceptible to attack by the surface microfungi[10].

In contrast, sixteen fungal species isolated from fruit surface were not observed to cause fruit rot during the survey period (Table 2). However, on being inoculated artificially, as many as ten fungal species were able to develop rot in apple fruits cv. Red Delicious (Table 2). It was noted that this cultivar was highly susceptible to *Trichoderma koningii* Rifai; susceptible to *Penicillium citrinum* Thom. and *P. purpurogenum* Stoll, moderately susceptible to *Fusarium sporotrichioides Sherb* and tolerant to *Acremonium strictum* W. Gams., *Aspergillus recurvatus* Thom., *Chaetomium globosum* Kunze ex. Fries, *Cladosporium oxysporum* Berk and Cult, *Emericella nidulans* (Eidan.) Vuill and *Paecilomyces variotii* Bainer (Table 2). This shows that fruit surface is an important habitat, which influences the occurrence and development of rot. Some of the fungal spores present on the fruit surface may colonize the host on getting a suitable microhabitat while some get killed due to unfavourable conditions or due to antagonistic effect of other microorganisms present on the surface. Similar observations have been made by a few other researchers while working on the surface microfungi of other fruits[4,5]. This investigation proves that many of the market diseases of apple cv. Red Delicious are initiated by the concentration of fungal spores present on the surface itself.

However, in contrast to the group of rot producers, six surface inhabiting microfungi viz. *Aspergillus fumigatus* Fresenius *A. terreus* Thom., *Memnoniella levispora* Subram., *Scopulariopsis brumtii* Salvant-Duval, *Trichurus spiralis* Hasselbring and *Verticillium lecanii* (A. Zimmerm) Viegas were not able to cause fruit rot of cv. Red Delicious when inoculated artificially (Table 2). Probably they did not possess the specific macerating enzymes necessary for pathogenesis. Among these, *A. fumigatus Fresenius* is well-known pathogen of animals and humans causing aspergilloma[11] and in addition is also reported to occur as a storage fungus causing deterioration of some fruits and vegetables[8,12]. Similarly, *A. terreus* Thom. is also a zoopathogenic mould but few isolates are even reported to cause fruit rot of harvested grapes[5] and guava[13]. *Trichurus spiralis* Hasselbring has also been earlier isolated as fruit and vegetable rot causing organism[12]. But the isolates obtained from surface of apple cv. Red Delicious were not capable of producing fruit rot. This proves that different isolates of the same species may have a different pathogenic potential.

All these facts lead to the conclusion that a definite relationship exists between surface myco- population and post-harvest fruit rot of apple cv. Red Delicious. The extent of deterioration may depend upon the population of inoculum of particular species, harvesting procedure practiced, physiological capabilities of the surface microfungi and the nutritional qualities of the fruit.

Acknowledgements

The authors wish to thank International Mycological Institute, U.K. and Indian Type Culture Collection, IARI, New Delhi for identification of some fungal isolates. The first author is also grateful to CSIR, New Delhi for giving financial help in the form of Senior Research Fellowship.

References

1. Sumbali G, Mehrotra R S. Qualitative and quantitative alteration in the sugar content of some pome and stone fruits induced by post-harvest spoilage fungi. *J Plant Dis Prot* 1981; **88** : 759-763.

2. Dennis C. Post- harvest pathology of fruits and vegetables. London: Academic Press, 1983 1-275.

3. Dennis C. The microflora of the surface of soft fruit. In: Preece T F, Dickinson CF editors. Microbiology of aerial plant surface. London: Academic Press, 1975. 219-430.

4. Kalafatoglue H, Karepinar M. Investigation of spoilage microflora in selected apple cultivars during storage. *Universitori Ziraat Dergisi* 1989; **26** : 347-356.

5. Singh J P, Kainsa R L. Microbial flora of grapes in relation to storage and spoilage. *Indian Phytopath* 1983; **36** : 72-76.

6. Tomkins RG, Trout S A. The use of ammonium salts for the prevention of green mould in citrus. *Jour Pomol Hort Sci* 1931; **9** : 257-264.

7. Singh YP, Sumbali G. Pre-harvest mycobial population of Indian jujube fruits (*Ziziphus mauritiana* Lamk.) and their implications in post-harvest pathogenesis. *Mycopathologia* 1998; **142** : 77-80.

8. Sumbali G, Badyal K. Relationship between fungal air-spora of fruit shops and incidence of fruit rots. *Indian Phytopath* 1991; **44** : 214-218.

9. Singh YP, Sumbali G. Performance of apple and pear fruit cultivars to post-harvest fruit rot fungi. *J Mycol Pl Pathol* 2004; **34(3)** : 914-916.

10. Dasgupta MK, Mandal NC. In: Dasgupta MK, Mandal NC, editors. Post - harvest Pathology of Perishables. New Delhi and Mumbai: IBH Publishing Co Pvt Ltd. 1989, 1-623.

11. Bulmer GS, Fromtting RA. Pathogenic mechanisms of mycotic agents. In: Howard DH editor, Fungi pathogenic for humans and animal. New York : Marcel Dekker INC, 1983. 1-59.

12. Sharma SK, Sumbali G. Post- harvest fungal decay of some vegetables from north India. *Indian J Mycol Pl Pathol* 1993; **23** : 241-246.

13. Madhukar J, Reddy SM. Incidence of mycotoxins elaboration in guava fruits. *Geobios New Reports* 1992; **11**: 188-189.

Proceedings of the National Academy of Sciences India

Section B: Biological Sciences

Instructions to Authors

A. What to submit

The journal publishes reviews or papers in any area of biological sciences. Special issue of the journal on proceedings of National and International Conferences/Symposia can also be published with one or more of the organizers as Guest Editors. At least one of the Guest Editors should be Fellow of the Academy. Guest Editors shall be responsible for ensuring quality and proper refereeing of the papers.

1. **Review Article:** It should be a critical review highlighting present status and an overview of the past work on a topic of current interest. It should help either in initiating research activity in that particular area or in increasing the comprehension of the current challenges. The contributors should preferably submit a brief pre-concept note or a two-page summary to the Chief Editor before preparing the review. If the review is accepted for publication, Rupees two thousand would be given to the 'corresponding author' after its publication to cover expenses towards its preparation. A review article should not exceed 30 printed pages (word limit 10,000).

2. **Original Article:** It should give results of the original research work done by the authors. Ordinarily, such articles should not exceed 12 printed pages (word limit 3,600).

B. Who can submit?

1. Investigators (Indian or Foreign) are welcome to submit manuscript for publication.

2. Fellows of the National Academy of Sciences, India can forward quality papers in their research field for publication in the journal. The name of the communicating Fellow would be printed in the paper as "Communicated by .... FNASc"

   The communicating Fellow should certify that:
   a). The forwarded paper is in his/her field of specialization.
   b). He/she has read the paper and reviewed it carefully. The Editorial Board, however, may get the paper reviewed by another referee if considered necessary.

C. Whom to submit

Original plus two hard copies of the manuscript should be sent to the Managing Editor (Biological Sciences), Proceedings of the National Academy of Sciences, India, 5 Lajpat Rai Road, New Katra, Allahabad-211 002, India. Electronic version (CD or 3.5" floppy disk) of the final copy would be required when the paper is accepted for publication.

D. How to submit

Authors may submit the manuscript directly or through a Fellow of the Academy as described above.

E. Format of the manuscript

1. The Proceedings is published in 'two column format' on A4 page.

2. The manuscript should be typed double spaced in 12-font size (Arial / Times / Times New Roman) on one side of good quality A4 size paper with one-inch margins. Insert hard returns only at the end of paragraphs. Page number should appear in the upper right hand corner of each page, beginning with the title page. File format for the text are MSWord, WordPerfect or LaTeX. Figures may be submitted as TIFF, EPS, PS, GIF, JPEG or PPT files.

Title Page

a). **Title** of the papers should be in **bold** (14 font size) and centered near the top of the first page.

b). **Author's names** should be in **bold** while **affiliations** should be in *italics*. Authors who are Fellows of the Academy should write FNASc after their names.

Arrangement of the text

a). The text of the paper should be arranged into Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgements and References.

b). **Abstract** (about 150 words) should be in **bold.**

c). **Keywords** [maximum 5, each separated by comma (,)] and a **Short running title** (maximum 50 characters including spaces) should follow the **Abstract**.

d). All **Figures** should be numbered consecutively using Arabic numerals. Line drawings and inscriptions should be legible. In case of scanned figures/line drawings, minimum resolution of 800 dpi should be used. All figures should be labeled on the back to indicate figure numbers, top margin and authors. Legends should be brief, self-explanatory, typed on separate page and placed at the end of the text.

e). **Tables** should be numbered consecutively using Arabic numerals, have self-explanatory title and submitted separately from the text. Footnotes to tables should be indicated by superscript lower-case letters. Abbreviations should be explained in the footnotes.

f). **References** should be indicated in the text by superscript Arabic numerals. The list of references should be arranged in order of their occurrence in the text. References should be given in the following style:

Journal article:

Khosla P, Bhargawa S, Singh J, Srivastava RK. Antinociceptive activity of *A. indica* (Neem) in rats. Indian J Pharmacol 2000;32:372-374.

Book:

Dhawan BN, Srimal RC. Laboratory Manual for Pharmacological Evaluation of Natural Products. First Edition, Trieste: International Center for Science and High Technology, 1997.

Book Chapter / Paper in Conference Proceedings:

Sriramana K, Nagaraju J. Conservation of biodiversity in genomic era. In: Tandon P, Sharma M, Swaroop R, editors. Biodiversity Status and Prospects. New Delhi: Narosa Publishing House, 2005. pp. 150-168.

Abstract in Conference / Symposium:

Singh MM, Gupta CM. Contraceptives from Natural Sources: Experiences and prospects. Proceedings of the Symposium on Expanding Contraceptive Choices: International and Indian Experiences and their Implications for Policies and Programmes, 2003, 7-8 December, Mumbai, India.

Dissertation:

Mehra PS. A clinical study on the effect of Amrita-Pippali-Nimba Yoga in cases of NIDDM with special reference to the role of Agni and Ojas. MD (Ay). Thesis. Banaras Hindu University, Varanasi, 1999.

Patent:

Srivastava N, Singh MM, Ray S. A process for the preparation of tertiary aminoalkoxy derivatives of substituted diaryl-5,6,7,8-tetrahydronaphthyl methane and their salts useful as fertility regulating agents. Indian Patent 187178, Central Drug Research Institute, Lucknow, 1998.

Website:

URI of the website should be mentioned e.g., www.nasi.org.in

Abbreviations of the names of journal/periodicals should conform to those given in the World list of Scientific Periodicals.

F. Preparation of Electronic version (CD or 3.5" floppy disk)

Ensure that your manuscript files are virus-free. Use only a new CD or floppy disk. Write the following information on the disk label:

1. File name(s)
2. Name of software and version used
3. Name of corresponding author
4. Dispatch date

G. Miscellaneous

1. All papers will be reviewed by the Editorial Board or External referees.

2. If a revision is recommended, three copies of the revised manuscript must be returned within two months of receipt of referees comments, otherwise it would be processed as

a fresh manuscript. The covering letter should indicate the changes made in response to referees' comments.

3. Upon acceptance of the paper, authors will be asked to transfer copyright of the paper to the publisher. This transfer will ensure wide dissemination of information.

4. Corresponding author will receive galley proof of the paper from the Academy's Editorial Office. Only typesetting errors can be corrected. No change and/or addition to the accepted manuscript will be allowed. In exceptional cases, a short post-script may be permitted. The corrected proof must be returned within a week of its receipt, otherwise the paper will be published with the corrections done by the Editorial Office.

5. Manuscripts not accepted for publication will not be returned unless specifically requested by the corresponding author.

6. Twenty-five offprints will be provided free of cost to the corresponding author. Purchase of additional offprints should be ordered on the form accompanying the proof.

SUBSCRIPTION RATE OF THE JOURNALS

Annual Subscription for both Sections (Sec A – Physical Sciences and Sec B – Biological Sciences) : Rs. 500.00; **for each Section** Rs. 250.00; **Single Copy :** Rs. 100.00, **Foreign Subscription** : (a) for one Section: US $100, (b) for both Sections US $200. **(Air Mail charges included in foreign subscription)**

Proceedings of the National Academy of Sciences India

(Section B: Biological Sciences)

DECLARATION AND COPYRIGHT TRANSFER FORM
TO BE SIGNED BY ALL THE AUTHORS

I/We, the undersigned author(s) of the manuscript entitled ....

....

.... hereby declare that:

a). The above manuscript has not already been published in part or whole (except in the form of abstract) in any journal or magazine for private or public circulation and is NOT under consideration for publication elsewhere.

b). No copyright material has been used/necessary permission has been obtained from the copyright holder for inclusion of such material.

c). Ethical clearance for experimentation has been obtained/was not required.

d). In the event of its acceptance, I/we agree to transfer copyright to the **Proceedings of the National Academy of Sciences India.**

e). I/We have read the final version of the manuscript and am/are fully responsible for its contents.

f). The work included in the manuscript is my/our own contribution.

g). No one who has contributed significantly to the work has been denied authorship and those who helped have been duly acknowledged.

h). Institutional permission, if applicable, has been obtained.

i). I/We agree to the authorship of the article in the following sequence:

	Author's name	Signature with date
1.	______________________	______________________
2.	______________________	______________________
3.	______________________	______________________
4.	______________________	______________________
5.	______________________	______________________
6.	______________________	______________________

NOTE

1. No addition, deletion or change in the sequence of authors is permitted.
2. If the authorship is contested, the manuscript may be returned or kept in abeyance till the issue is resolved.
3. This form may be photocopied and used.

Contents of the forthcoming Special Issue on Wetland Ecology

The journal is being sent regularly to the following Abstracting Agencies for Indexing:Thomson Scientific, Philadelphia, USA; Elsevier Bibliographic Database, Amsterdam;Chemical Abstract-Service, Colombus, USA; INGST-CNRS,France; Springer Verlag, Germany; BIOSIS,Philadelphia,USA; Zoological Records,UK and others. For free access to the journal,pl.see the website-http://nasi.iiita.ac.in/library